# THE ROLE OF ENDOGENOUS HYPERLIPEMIA IN EXPERIMENTAL ATHEROSCLEROSIS

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1969

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# THE ROLE OF ENDOGENOUS HYPERLIPEMIA IN EXPERIMENTAL ATHEROSCLEROSIS

A Thesis Submitted
in Partial Fulfillment
of the Requirements for the Degree
of Doctor of Medicine

by

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1969

To my Father, Wife, and Unborn Child

ACKNOWLEDGEMENT

I am deeply indebted to Dr. Levin L. Waters for his constant encouragement and superb teaching. He has always been available for constructive criticism, objective evaluation and genuine support.

I am also grateful to Edward Iannucci, Peter Integlia, Helen Cavallero, Sharon Williams and my wife Ann for many hours of technical assistance and manuscript preparation. This thesis was in part supported by U.S.P.H.S. grants.

CONTENTS

## INTRODUCTION

More lives are claimed in our country by atherosclerosis and its complications than by any other disease process. In order to prevent atherosclerosis, investigators have been endeavoring to understand its pathogenesis. Various experimental models have been developed for this purpose. Saltykow[120a], in 1908, and Ignatowski[62], in 1909, first discovered that milk, meat and egg diets could produce atherosclerotic lesions in rabbits. Cholesterol feeding in one or another form has since been the primary method of induction of the characteristic arterial changes.

More recently the role of endogenous lipids rather than dietary cholesterol in the process of atherogenesis has been considered. Certain questions could then be asked: where do atherogenic lipids originate in the body? Which types circulate in the bloodstream? Why do they localize in arterial walls at certain regions? Are endogenous lipids involved in the atherogenetic process?

During the past decade workers have raised these and similar questions. They have become increasingly concerned with carrier states of lipids in the bloodstream (lipoproteins)[45]. Distinct combinations of plasma lipoproteins have been documented in human population groups chiefly by Fredrickson and his associates at the National Heart Institute[38,39,81-3].

The following investigation is a study of the role in the experimental atherosclerotic process of induced <u>endogenous</u> lipidemia. Included are anatomic studies of resultant lesions and a consideration of the chemical nature of lipoprotein types involved.

## LIPOPROTEIN CLASSIFICATION

Fredrickson and his associates at the National Heart Institute have been studying human serum lipoprotein disorders for several years. In 1965[38] and again in 1967[39] and 1968[83] they published extensive reviews of current findings. They have described five types of "essential" human hyperlipemias, readily identifiable by their electrophoretic patterns. Many of the characteristic patterns also appear secondary to other disorders such as biliary obstruction, hypothyroidism and diabetes.

The first pattern, Type I, is known as hyperchylomicronemia or severe exogenous hyperlipemia. Of less than fifty known patients reported, all have had an early onset. Attacks of abdominal pain are frequent complaints, and no diabetes or severe atherosclerosis has been observed in this group. Deficiency of "lipoprotein lipase" leads to low post-heparin lipolytic activity, and numerous chylomicrons in fasting overnight plasma samples, even while these individuals are on a low-fat diet.

"Essential familial hypercholesterolemia", Type II hyperlipemia, is the most common. Normal types of beta lipoprotein concentrations are increased, and a moderate increase in pre-beta lipoproteins is noted. Homozygous individuals may have fatal atheromatosis in early childhood. Coronary artery disease is common. Patients with hypothyroidism often display this pattern of lipoproteins.

The Type III pattern individuals discussed by Gofman in 1954[45a] may be recognized clinically by their palmar xanthomas. These patients often have a family history of diabetes and have a greatly enhanced incidence of occlusive peripheral vascular and coronary artery disease. Their

turbid plasma contains greatly increased quantities of cholesterol and glycerides. A broad beta band including pre-beta components distinguishes the electrophoretic patterns in this group.

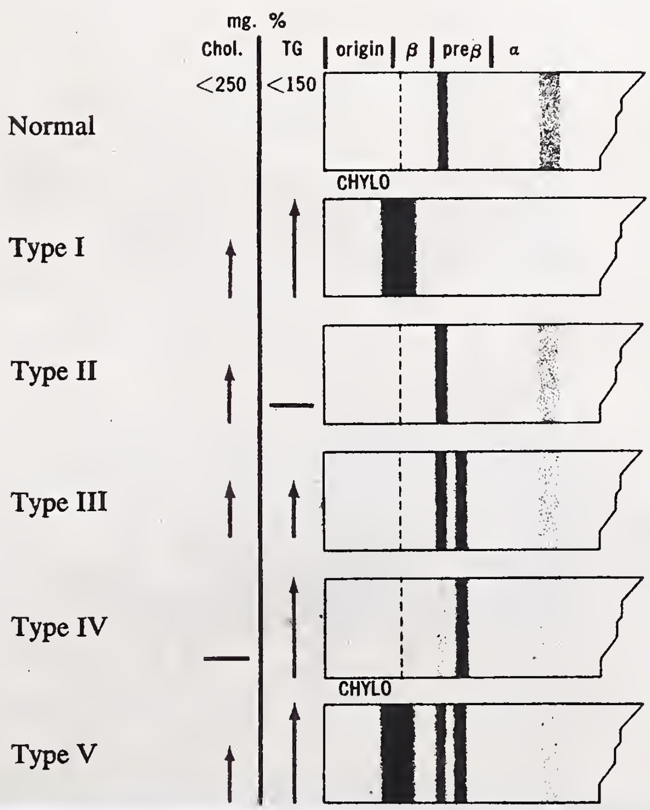
Greatly elevated pre-beta lipoproteins are observed in Type IV patients. Too many glycerides are produced by their livers, and obesity and diabetes are the rule. Similar patterns are seen in patients with hypothyroidism, glycogen storage disease, diabetes mellitus, nephrotic syndrome, and in some individuals after myocardial infarcts.

The final group of hyperlipemic patients are classified as Type V, a combination of "endogenous and exogenous" hyperlipemia patterns. These persons experience bouts of abdominal pain often in their third decade but they usually have diabetes mellitus and/or a strong family history.

# CLASSIFICATION

## BLOOD CHEMICAL AND LIPOPROTEIN PATTERNS*

### Primary or Familial Hyperlipidemia

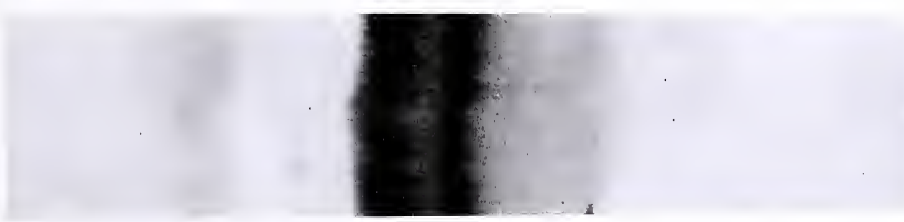


EXAMPLE OF A PATIENT WITH A SECONDARY HYPERLIPEMIA

W. S. is a 52-year-old white German male who has a fifteen year history of diabetes mellitus requiring about 75 units of N.P.H. insulin per day. He has had two right sided C.V.A.'s with regaining of most function, has moderate diabetic retinopathy with some blurred vision, no xanthelasma, only mild hypertension (B.P. 150/95), rather massive obesity (wt. 230 lbs., ht. 65 in.), calcified abdominal aorta, intermittent claudication, no angina pectoris, warm extremities with palpable pulses, and one flight dyspnea without jugular venous distention or peripheral edema. In October 1968 his fasting morning blood showed: gross lipemia-milky appearance

total cholesterol 249 mg.%
free cholesterol 36%
fatty acids 18.3 meq./1
tri-glycerides 680 mg.%

His serum lipoprotein electrophoresis (courtesy Dr. R. Scheig) showed a prominent beta and markedly increased pre-beta band consistent with Fredrickson's Type III pattern.



The patient is now on a four-month trial of Atromid-S (Clofibrate), and repeated dietary counseling has been given. Prior control with diet alone was unsatisfactory.

## EXPERIMENTAL - MATERIALS AND METHODS

As indicated in the Introduction, the objective of this study was to test the role of endogenous lipidemia in the pathogenesis of experimental atherosclerosis. It is well known that local, acute lesions of animals' arteries, however produced, may be readily modified to changes of the arteriosclerotic type by a cholesterol-rich diet. Therefore the plan of the present experiments was to produce such lesions in the arteries of rabbits and to test the ability of induced endogenous lipidemia to modify the basic lesions in a similar fashion. The goal was to obtain a combined experimental procedure which yielded frequent arterial lesions and a significant lipemia in the majority of animals.

Seventy-four adult New Zealand white rabbits, randomly sexed, weighing 2-5 kg. were maintained on a low-fat (2 percent) diet (Purina rabbit chow), with drinking water *ad libitum*, in individual wire mesh cages in the Yale animal care unit. Blood samples were drawn with sterile disposable syringes from either the femoral vessels or via cardiac puncture. Drugs were administered (except where noted) via the marginal ear veins. Drugs employed were as follows:

Epinephrine 4 ml. vials 1:1000 (Parke-Davis)
Levophed(R) Bitartrate 0.2% 4 ml. vials (Winthrop)
Tyramine Hydrochloride Powder (Nutritional Biochemicals)
Horse Serum, Pooled Sterile (Baltimore Biological)
Pituitary Powder, Beef Defatted and Whole Beef
(Nutritional Biochemical)
Triton WR 1339 (Ruger).

Initial experiments on vascular injury were conducted with epinephrine. Five rabbits were given different doses intravenously. Two animals survived the first series of injections. Only minor histological changes, mainly edema of many tissues, were seen up to one week after the experiments.

Levophed(R) (Nor-epinephrine) was employed intravenously for two additional rabbits, but aside from the interesting clinically observable hemodynamic effects (noted later), no significant histologic lesions were seen.

Two more animals were subjected to tyramine injections following the method of Duff[32]. Again, significant arterial lesions could not be demonstrated. (Our early experiments are summarized on Chart 1).

It was then decided to use the experimental hypersensitivity method of Rich and Gregory[111-113] to induce vascular injury. Rabbits were given 10 cc./kg. body weight of sterile pooled frozen horse serum on three successive weekly occasions. Many of these rabbits also received whole dried beef pituitary extract to induce an endogneous lipemia according to procedures outlined by both Rudman[120] and by Kellner[70] for raising serum lipid concentrations. Unfortunately these methods did not yield arterial lesions consistently although sporadic lesions and lipemias were noted. (See Chart 2 for a summary of the experimental findings).

Finally Triton WR 1339, a non-ionic detergent was utilized to induce readily repeatable endogenous lipemias. A single 10 cc./kg. intravenous injection of 10% sterile Triton solution was administered under sterile conditions (according to the technique of Courtice and Schmidt-Diedrichs[24,25] and of Kellner[69].

A modification of the technique of Kelly[71,142] was used to injure hyperlipemic rabbits' aortas _in vivo_ by freezing. Instead of ethyl chloride spray aortic segments were frozen by the application of dry ice directly at laparotomy.

Anesthesia for aortic freezing experiments was 15-20 cc. of Nembutal in a single dose supplemented when necessary by open drop ether inhalation. Surgical operations were conducted under aseptic conditions. Aortic freezing was performed as follows: the rabbit was immobilized in a supine position by tying all four extremities and administering a single dose of intravenous Nembutal. After shaving from midchest through abdomen, the ventral skin was cleansed with standard Zephiran solution. After checking for regular unobstructed respirations and keeping an ether cone ready, a generous midline abdominal incision was made. The peri-aortic connective tissue was dissected away and the aorta was mobilized for 2-4 cm. well below the origin of the renal arteries. A kelly clamp was inserted behind the aorta, and the vessel was gently lifted, spreading the clamp's jaws 1-2 cm. apart, until the pulsations ceased. At this point the flat edge of a suitably-sized piece of dry ice was applied uniformly along the raised aortic segment for 30 seconds. The aorta was then allowed to fall back into place and the kelly clamp removed. Five to ten cc. of sterile saline at room temperature was then poured over the frozen vessel. Often a primary aneurysmoid bulging was observed before the segment again began to pulsate and regain its tone. A fine silk ligature was inserted transversely in the left psoas muscle mass to mark the region of injury. The abdomen was closed with silk in three layers. The skin was closed with a continuous inverted mattress silk suture. Rabbits were closely observed until they recovered, usually in about 2-4 hours. They were then replaced in their original cages in the animal room, with drinking water ad libitum, and, as before, Purina rabbit chow.

Rabbits were sacrificed at appropriate times using 4-5 cc. intravenous Nembutal. Organs were fixed in 10% formalin for at least 24-48 hours. Tissues removed and examined included: brain and pituitary (occasionally), heart, lungs, aorta (intact, when possible), a portion of liver, adrenals, kidneys, spleen, abdominal fat and musculature. Animals dying overnight were not included in the study unless found within 1-2 hours of death.

Histological preparations were made both by frozen section followed by staining with Sudan IV for fat, and by routine hematoxylin and eosin staining for permanent sections.

Black and white photomicrographs were taken with an American Optical binocular microscope fitted with a Kodak Color Snap 35 mm. camera. Kodak Plux-X film (ASA 125) was used with a Wratten 58 (green) filter.

Serum lipid determinations were conducted as follows: fatty acids according to Man and Gildea's modification of Stoddard and Drury[87] followed by the method of Stern and Shapiro[138] when Triton was found to interfere with fatty acid precipitation in the former, phospholipids according to Hawk and Oser[51], total and free cholesterol following Schoenheimer and Sperry[127]; triglycerides were obtained by the following calculations:

1-convert cholesterol and phospholipids to meq./liter.

a) $\frac{\text{total-free cholesterol}}{38.6}$ or if no free cholesterol

total cholesterol x 0.0186

b) phospholipids x 0.58

2-triglycerides (meq./1/)=fatty acids (meq./1.) -

[chol. (total-free) (meq./1.)+phospholipids (meq./1.)]

Blood samples were immediately centrifuged and sera were refrigerated if not analyzed promptly.

Electrophoretic patterns were obtained by a cellulose acetate method (Beckman) courtesy of John Kelly[72]. The serum lipoprotein electrophoresis pattern of W.S., the patient reported herein, was obtained through the kindness of Dr. R. Scheig, Department of Medicine, Yale-New Haven Hospital.

Finally, hyperlipemic serum (removed from rabbits both twenty-two hours and four days after an injection of Triton) was injected aseptically through a 30 guage hypodermic needle into the superficial central portions of the corneas of anesthetized rabbits: these animals were then sacrificed at appropriate times and their corneas removed for both gross and microscopic observations.

## RESULTS

The five rabbits given intravenous epinephrine showed no changes indicative of recent gross arterial injury. Such acute findings as petechial abdominal wall hemorrhages, pulmonary edema, generalized congestion and isolated regions of foam cells in peri-aortic abdominal arterioles were noted. The latter change was considered to be spontaneous. Only rabbit WA-4 showed a significant finding: A left ventricular apical aneurysm with subendocardial hemorrhages.

The two animals receiving Levophed(R) demonstrated no experimentally induced lesions, but one was soon able to tolerate huge doses of Levophed(R) with comparatively slight clinical effect. Bradycardia lasting about three minutes was noted after each dose.

One percent tyramine hydrochloride was given to two other rabbits: the first tolerated daily 7.5 cc. doses for six days and had no remarkable post-mortem changes. WA-9, however, succumbed with cardiac arrest following grand mal seizures minutes after a single dose of tyramine. No remarkable experimentally induced post-mortem findings were noted. (See Chart 1 for a summary of these preliminary experiments.)

Rabbits 10,12-42 received beef pituitary extract in doses of 100-200 mg. per animal subcutaneously in one of several forms (see Chart 2) in attempts to induce significant endogenous hyperlipemia according to procedures outlined by both Rudman[120] and by Kellner[70]. In addition, numbers WA-10-12, 21-24, 34-37, and 39-42 received one to three doses of sterile pooled horse serum intravenously to produce concomitant vascular injury following the technique of Rich and Gregory[113]. Doses of pituitary above 500 mg. occasionally yielded gross lipemia but more than 800 mg.

was toxic within twenty-four hours. Fatty acids, only, were sometimes elevated to 100-400 meq./l. No consistent experimentally induced vascular lesion was noted, and the narrow margin of safety with pituitary extract made the above methods unsatisfactory.

Intravenously administered non-ionic surface-active detergents were found by Kellner[68] and again by Cornforth[23] in 1951 to yield sustained endogenous hyperlipemias. It was decided to combine the use of detergents and direct aortic freezing, according to Taylor (1950)[142] and Kelley et. al. (1952)[71], to obtain regularly reproducible hyperlipemia concomitant with known vascular injury. With one intravenous dose of ten percent sterile Triton solution grossly evident lipemias were consistently obtained. The chemical effects on the blood of a single 7-11 cc. dose were evident within a few hours. Sera remained lactescent for approximately six days and chemical hyperlipemias were evident for about eight to ten days (see Charts 5 through 8). Total and free cholesterol rose from average baseline values of 69.3 mg.% (range 33-161 mg.%) and 21.3 mg.% (range 9.1-48 mg.%) to peaks of 470-740 mg.% and 335-490 mg.% respectively between three and seven days. Slightly elevated values were still evident two weeks after the single dose of Triton.

Fatty acids abruptly increased from average baseline amounts of 11.0 meq./l. (range 4.0-18.0 meq./l.) to values of 125-290 meq./l. within 48-72 hours followed by a steady decline to control after nine to thirteen days. Triglycerides followed in like fashion. Triton was found to interfere with precipitation of fatty acids in Stoddard and Drury's modified procedure, consequently, Stern and Shapiro's method as indicated was employed with success. A comparison of the two procedures performed to

verify accuracy on sera not containing Triton may be found on Chart 4. Close agreement of determinations was noted.

Phospholipids peaked in two to five days in the range of 22-55 mg.%. Pre-Triton levels of 5.2 mg.% (range 2.5-8.6 mg.%) were approximated after two weeks.

It was remarkable that every rabbit given a single dose of about 250 mg. Triton responded with a predictable rise in fatty acids (and, therefore, triglycerides), followed by steady elevations in both phospholipids and total and free cholesterol. The 22 hour pooled centrifuged sera with moderate total cholesterol elevations (from rabbits WA-57 and WA-58) were found to invoke only slight corneal foam cellular reactions in rabbits WA-61 and WA-62. (see Figures 1 and 2).

By contrast four day post-Triton serum was noted to induce more corneal reaction with moderate numbers of foam cells and lipid observable between and apparently within collagen fibrils. (WA-65 and 66). This serum contained high amounts of cholesterol and less lipid in the form of triglycerides. Corneal foam cellular reactions obtained by Grauer[48] with sera containing greatly increased cholesterol in the range of 100 mg.% were significantly more intense (Figure 4). It would appear, then, that the high cholesterol lipid fractions exert a greater atherogenetic effect in the rabbit corneal models than do other lipid types.

Frozen sections from the aortas, coronary, and pulmonary arterioles of rabbits sacrificed within 24-48 hours after a single Triton injection, however, demonstrated significant sudanophilic material. Increased uptake of lipid was noted in selectively injured aortic segments. Grossly fatty livers and peri-aortic postoperative inflammation and hemorrhage were seen.

After 4-5 days the livers were still markedly fatty and blood cholesterol was elevated substantially. Segments of aortas formerly subjected to freezing in the first 24 hours of lipemia showed extensive periadventitial hemorrhage and granulation tissue with polymorphonuclear leucocytes and mononuclear cells, medial hyalinization and necrosis, and subintimal separation and proliferation (Figures 5-8,19). Higher power views revealed the subendothelial space accumulating monocytes and small foam cells (Figures 8,9,12,13). So called "tadpole" cells were observed in the aortic lumen (Figures 10,11) and foam cells were seen migrating through the internal elastic lamina (Figure 15). Lipid was also present between intimal elastic laminae in intracellular droplets (Figures 8,9,13-18).

Three types of controls were examined. Unfrozen, unmanipulated portions of the aorta, and other arteries in animals subjected to either Triton or to aortic freezing were scrutinized for signs of the effects of Triton. No lipid infiltration was noted in uninjured aortic segments. In addition, aortas from rabbits WA 68-72 (animals subjected solely to Triton and periodic blood collection) showed no regions of lipid deposition. Pulmonary arterioles, however, frequently contained sudanophilic intimal and medial deposits sometimes with foam cellular responses.

Two rabbits (WA-58 and 59) were given intravenous sterile normal saline instead of Triton and subsequently underwent aortic freezing. Injured aortic segments after eleven days contained medial calcification, subintimal proliferation and only trace amounts of lipid (Figures 20,21). Pulmonary arterioles showed subintimal swelling without any stainable lipid. Lungs were essentially normal with some regions of isolated congestion. Heart, liver and kidney were considered normal. Rabbit WA-53,

that was sacrificed at the same time, but after both Triton and freezing, proved to have similar aortic findings but with the addition of slight liquefaction and lipid in the inner media, and small amounts of remaining hepatic lipid.

WA-55 and 56 received Triton but their abdominal aortas were touched with aneurysm hooks instead of dry ice for thirty seconds. The remainder of their treatment was identical to that of rabbits whose aortas were frozen. After one week, the aortas had thickened folded internal elastic lamellae but no subintimal proliferation or stainable lipid. Peri-aortic arteriolar medial lipid was noted however. No stainable fat was seen in the livers. WA-55 had phlebitis of the inferior vena cava, and renal arterial intimal edema was noted in conjunction with focal round cellular renal infiltrates in WA-56.

Twenty days elapsed after the aorta of WA-49 was frozen. Virtually no hepatic lipid was noted, nor lipid in the aorta. Slight subintimal proliferation and extensive medial calcification and necrosis was observed (Figure 22). The lungs were of interest; they contained intimal proliferation of the pulmonary arterioles, and lipoid pneumonia was present (Figure 24) with hemorrhage, foam cells (Figure 25), mononuclear cells, and giant cells (Figure 26). (For comparison, Figure 23 illustrates a region of lung from WA-60, an animal receiving no Triton.) Finally, phlebitis of the inferior vena cava was evident. By comparison, lipid was noted in the aorta of WA-50 (Figure 27) twenty-eight days after freezing, and subintimal proliferation (Figure 27) along with widespread medial necrosis were seen (Figure 28). The liver still contained some lipid (Figure 29).

The final animals (WA-45 and 51) were sacrificed three months after a single injection of Triton followed by aortic freezing. One (WA-45) showed medial liquefaction and calcification with trace amounts of stainable lipid. Both rabbits had pulmonary arteriolar intimal proliferation. WA-45 had a myocardial infarction located in the interventricular septum.

An incidental finding was that rabbits given Triton suffered weight losses of 1-2 pounds in the first two weeks. They ate and drank during this period (although precise amounts were not measured) and they occasionally had diarrhea. Abdominal fat reserves were slightly reduced.

No glomerular foam cell lesions were noted (reported by Vidone and Lowman[148] in dogs). Our rabbits, however, received only single doses of Triton.

## DISCUSSION

### A. Spontaneous Arterial Lesions in the Rabbit.

Three types of spontaneous aortic lesions have been described in the rabbit. Israel, in 1881[65] named "chronic endo-aortitis deformans", a spontaneous medial degeneration and calcification. Quadri (1907)[109], Miles and Johnstone (1907)[92], Ophüls (1907)[97], Pearce (1908)[102], Lucie and Parisot (1908)[85], Hill (1910)[54], and Nazum et al. (1930)[95] found similar lesions but reported different incidences. Kesten[73] found aortic and iliac artery medial calcifications in 87% of eight month old rabbits. Atheromatous yellow plaques were seen in the adult aortic arch by Ophüls in 1907 and by Nazum et al. in 1930. Finally, a raised polypoid region consisting of variously-directed layers of smooth muscle cells in a mucopolysaccharide stroma was described by De Faria in 1955[28] and confirmed by Haust and More ten[50] years later.

In addition, spontaneous muscular pulmonary artery thickening with splitting of the internal elastic lamina, increased number of elastic fibers, and a narrowed vessel lumen were presented by Prior et al. in 1961[108].

As Haust and More indicate, however, the frequency of these lesions varies greatly according to observers, and all factors, such as strain background, living conditions (temperature, humidity, barometric pressure, availability of food and water, exposure to other species and each other and parasitic and infectious diseases), and age. In general, however, spontaneous <u>atherosclerosis</u> is rare in rabbits.[26]

B. Cholesterol Feeding Experiments in Brief.

In 1908, milk, eggs and meat were noted to be atherogenic in rabbits[120a]. Ignatowski[62] corroborated these findings in 1909. Many investigators have administered cholesterol and cholesterol-modified diets since that time. Reviews of progress in experimental atherogenesis using cholesterol feeding models appear periodically[26,160,22]. At this time prolonged cholesterol feeding in animals is the basic experimental model for human atherosclerosis.

Many difficulties are encountered in cholesterol feeding. Somatic afflictions of experimental animals[22], and long duration are two examples. Vascular lesions produced do not always occur in regions comparable to natural lesions in humans.

C. Endogenous Lipemias and Triton.

Perhaps the first experimental induction of a lipemia not related to feeding of exogenous lipids was reported in 1909 by Boggs and Morris[7]. They found rabbits subjected to daily bleedings of 25 cc. developed hyperlipemia after eight to sixteen days. Friedland's experience with desiccated thyroid will be mentioned later[40]. Reticulo-endothelial blockade with substances such as India ink has also led to lipemia.

Increased blood lipids are noted during exposure to cold, therapy with corticoids[114] and in individuals afflicted with the nephrotic syndrome[46,118,131]. Zarafonetis mentioned these as well as femoral fracture, protamine and diisopropylfluorophosphate for inducing lipemia. Cobaltous chloride led to similar blood lactescence in experiments reported by Caplan and Block[14] and by Brody[9]. Additional hyperlipemic conditions were reviewed by Scanu in 1965. Cushing's syndrome, essential xanthomatosis, excess growth hormone, increased catecholamines, obstructive jaundice and androgen influence were also mentioned.

Triton, a non-ionic detergent, was first associated with hyperlipemia by Hueper in 1944[61]. Triton in a 0.1 percent solution was administered both orally and intravenously to rabbits and was associated with severe toxic manifestations. Foam cellular lesions were noted in pulmonary arteries and aorta. The toxicity of Triton apparently deterred other investagators until, in 1951, Cornforth[23] found elevation of blood lipids after testing Triton for experimental tuberculosis therapy. Later that year, Kellner, Correll and Ladd[68,69] reemphasized the hyperlipemic effects of Triton.

From this point on, investigators wondered about the mechanism of action of Triton. Frantz[37], Friedman[41,42], Hirsch and Kellner[55,56,57], Kellner[70], Otway[98], Pawar[101], Pethica[103], Radding[110], Scanu[121-4], Schotz[128], Vidone[148], Zarafonetis[165,166] advanced theories about Triton's action in the plasma, reticuloendothelial system, liver and posterior pituitary. The present author believes its actions holding lipids in the plasma and inhibiting removal by the liver are particularly significant. Triton may interfere with specific enzyme systems and block reactions at membrane surfaces.

Triton-induced lipemia has a characteristic pattern. During the first few hours the serum contains increasing amounts of fatty acids until lactescence is noted. The next day cholesterol (both total and free) build up to values several times normal, even in animals maintained on low-fat diets. Triglycerides and fatty acids reach peak amounts on the second day and steadily decline, while phospholipids and cholesterol remain markedly elevated for about one week. Electrophoretic patterns of this lipemia demonstrate a pattern similar to Fredrickson's Type III; both beta and pre-beta fractions are increased.

D. Experimental Vascular Injury.

Interest in experimental production of vascular injury has steadily increased since the turn of the century. In 1903, Josué reported experimental aortic lesions after repeated injections of adrenalin(R)[66]. Waterman[149] noted medical necrosis and calcification of small muscular arterioles in 1908. Anitchkow[2] employed adrenalin(R) for vascular injury in 1913. Schmidtmann obtained selective focal arterial damage in 1929 using vitamin D overdosage[126]. Desiccated thyroid was found by Friedland to produce experimental arterial injury (cited by Anitchkow in E.V. Cowdry, 1933)[40].

Goldblatt[46] reported arteriolonecrosis in dogs with malignant hypertension secondary to induced renal ischaemia in 1938. Duff and Rich in 1936 used subcutaneous trypsin injections, Duff, again, in 1939[32] employed tyramine in rabbits and Waters, nine years[150] later obtained lesions using allylamine in dogs.

More recent chemical procedures for producing vascular injury have included: seven percent saline diets given to rats to induce nephrotic syndromes with edema, hypertension, anemia, lipemia, hypoproteinemia and severe arteriolar disease (1958), aminopropionitriles, according to Schwartz[130] (1959), and diethylstilbestrol injections that produced dissecting aneurysms and aortic ruptures in turkeys (1966)[134]. Norepinephrine[24,140,141] was noted to induce arterial and myocardial damage in patients.

Serum sickness has served as an elegant manner of inducing experimental vascular injury since studies by Fleischer et. al[36] (1931), Clark and Kaplan[17] (1937) and Rich and Gregory's well-known reports in 1942 and 1943[111-113].

Direct physical trauma to arteries has consistently resulted in vascular injury. Ssolowjew[136,137], in 1929, cauterized blood vessels. Juvenile rabbits' aortas were frozen by Taylor et. al. in 1950[142]. Kelly, Taylor and Hass[71] directly froze cholesterol-fed rabbits' adbominal aortas with ethyl chloride spray two years later. Prior and Hartman[106] scraped aortic intimas of hyperlipemic animals with a hooked needle and found increased atherogenesis in 1956. Williams, five years later[159], had similar results with only surgical mobilization of hyperlipemic animals' aortas, and Baumgartner et. al[4] and Gutstein et. al.[49] stretched aortas to obtain severe vascular disease. In 1968, Hoff[58] merely subjected aortas to brief ligation to observe changes.

A controversy exists in the literature regarding the ability of Triton to induce morphologic counterparts of atherosclerosis in experimental animals. Courtice and Schmidt-Diedrichs[25] reported no retained lipid or foamy lipophages in carotid arteries of rabbits injured by local infusions of heated saline. At the times of injury these animals had a Triton-induced hyperlipidemia. In contrast, Vidone and Lowman[148] found extensive free and foam-cellular intimal lipids in aortas and coronary and pulmonary arteries of dogs subjected to repeated injections of Triton alone. Grossly these arterial lesions appeared as fatty streaks. These dogs received no other form of experimental vascular injury.

The results of the present experiments would indicate that an experimental endogenous lipemia, similar to Fredrickson's Type III in humans, does indeed alter acute arterial lesions in rabbits to morphological sequences like those of early atherosclerosis in man. This evidence broadens our concepts of the role of lipids in atherogenesis and indicates

that the emphasis on study of exogenous dietary cholesterol in the process may have dealt with only part of the problem.

## SUMMARY AND CONCLUSIONS

Seventy-four rabbits on low-fat diets were employed in a study of experimental vascular disease with concomitant endogenous lipemia. The resultant lesions were considered in relation to human atherosclerosis and to the lipoprotein patterns present in the animals' blood. Preliminary experiments with epinephrine, norepinephrine, tyramine and sensitization with horse serum proved unsatisfactory methods for producing vascular damage. Injection of pituitary extract produced only occasional mild hyperlipemias insufficient in magnitude for our purposes. Also this procedure was very toxic to the experimental animals.

Direct physical trauma to intact abdominal aortic segments _in vivo_ provided predictable vascular injury with medial necrosis and calcification and subintimal proliferation. Spontaneous subintimal reaction in pulmonary arterioles was often seen.

Triton WR 1339, a non-ionic detergent, when administered as a single intravenous dose of 250 mg./kg. body weight led to significant elevation in plasma lipids; first triglycerides, then cholesterol (total and free) accompanied by phospholipids rose to amounts sufficient to produce grossly milky serum. Lipemias remained at peak levels for three to seven days and were approaching control levels in nine to fourteen days. Electrophoresis revealed serum lipoproteins in a pattern similar to Fredrickson's Type III described in patients. An example of a patient with a secondary Type III pattern was presented for comparison.

Histological preparations of aortas from animals with concomintant vascular injury and Triton hyperlipemias revealed aortic lesions with many hallmarks of human atherosclerosis including subintimal proliferation,

foam cellular accumulation with lipid deposits in the intima and media, medial necrosis and liquefaction with calcification, and periadventitial reaction. "Tadpole cells" (young foam cells) were seen in the aortic lumen and penetrating the internal elastic membrane. The membrane, itself, was seen accumulating lipid droplets within its cells, and one cell was photographed in mitosis. Pulmonary arterioles were noted to accumulate stainable lipid occasionally. Livers remained fatty for between one and two weeks after Triton. Lipoid pneumonia with giant cells and foamy alveolar macrophages was noted in some instances.

Rabbit corneas were used as sites for ancillary models of atherosclerosis following concepts and procedures outlined by Waters[154,155,157] and by Grauer[48]. Twenty-two hour, post- Triton serum produced little corneal stromal lipophagic reaction whereas four day, post-Triton serum containing high cholesterol values and moderately increased fatty acids yielded mild foam cellular responses. Even these reactions were less than the known marked responses in corneas injected with serum having very high cholesterol levels[48]. It would appear, then, that cholesterol fractions are more atherogenic, at least in the corneal models.

Aortic freezing and Triton injection provide a reliable and readily obtainable model with many characteristics of human atherosclerosis.

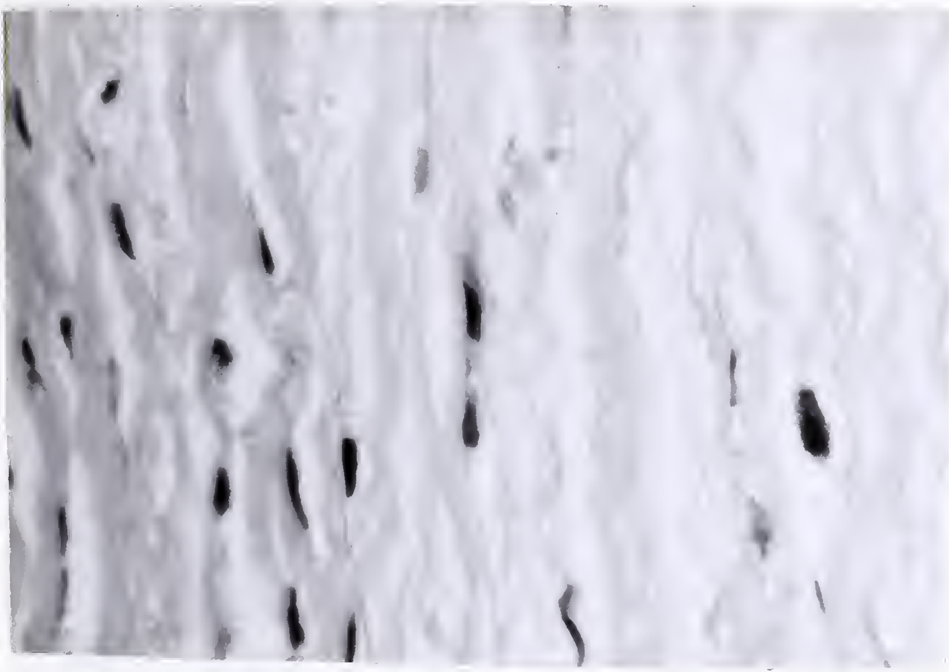


Figure 1. WA-61 rabbit cornea eight days after instillation of twenty-two hour post-Triton serum. Scarce foam cells. (H & E, 450x original magnification)

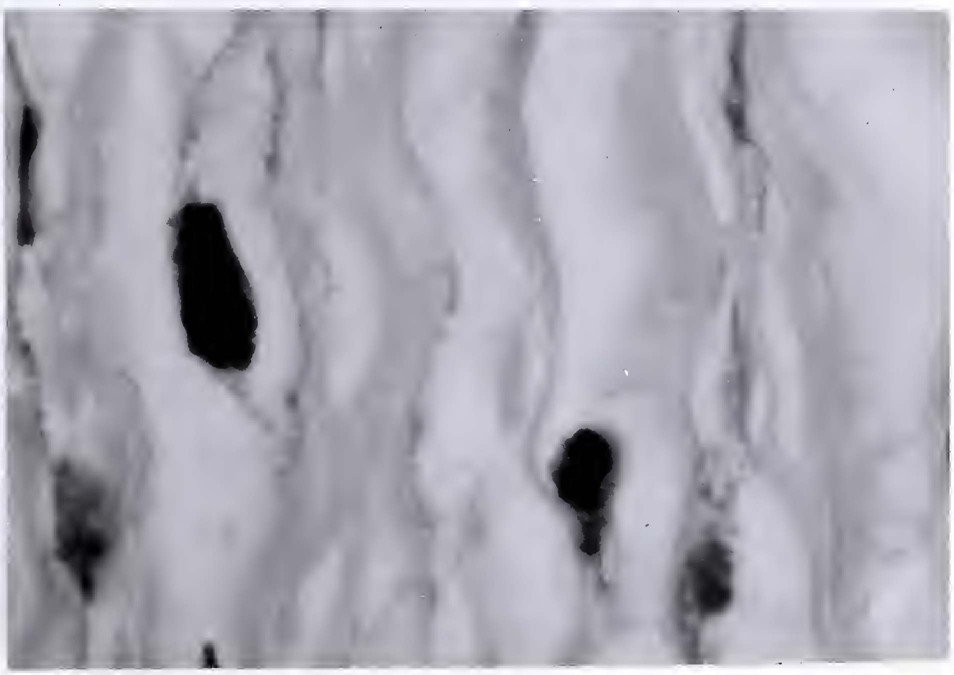


Figure 2. WA-61 cornea. Foam cells. (H & E, 1000x original magnification)

Figure 3. WA-61 cornea. Droplets of lipid within collagen fibrils and extracellularly.
(Sudan IV. Frozen section. 450x original magnification)

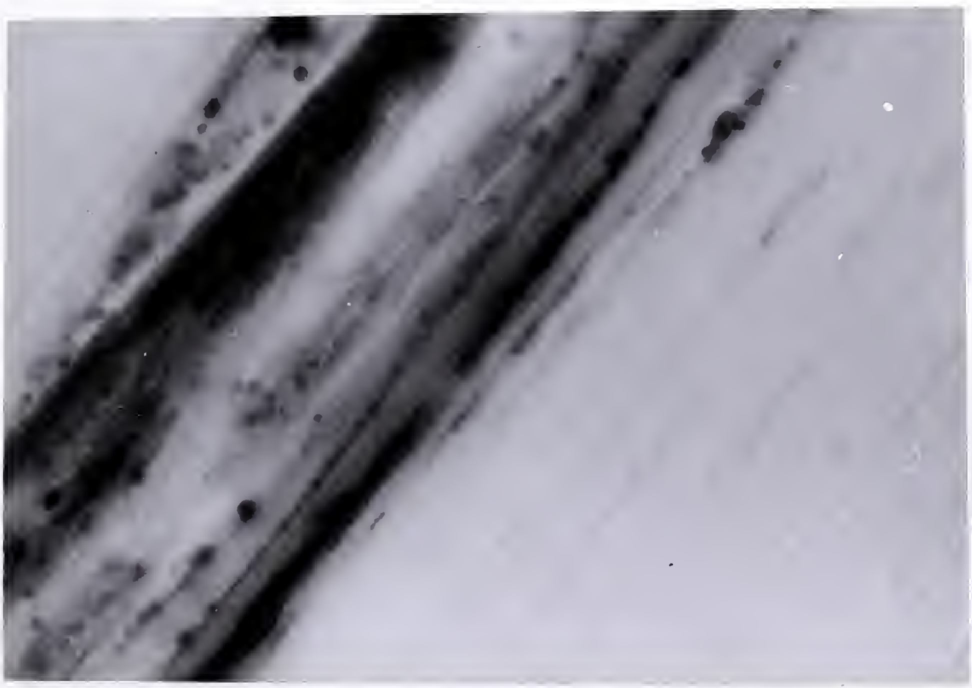


Figure 4. WLG-994-RC cornea six days after instillation of hypercholesterolemic rabbit serum. Abundant intra-and extracellular lipid.
(Sudan IV. Frozen section. 100x original magnification.
Courtesy L. Graver, 1968)

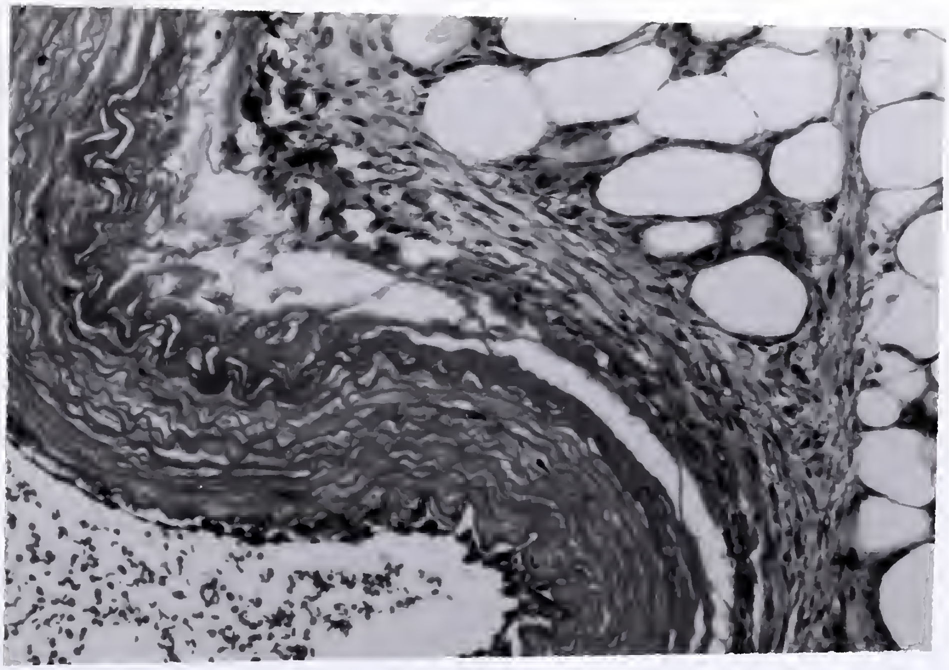


Figure 5. WA-44 aorta five days after single injection of Triton and four days after aortic freezing. Intimal proliferation, medial necrosis, periadventitial hemorrhage, granulation and fat. (H & E, 100x original magnification).

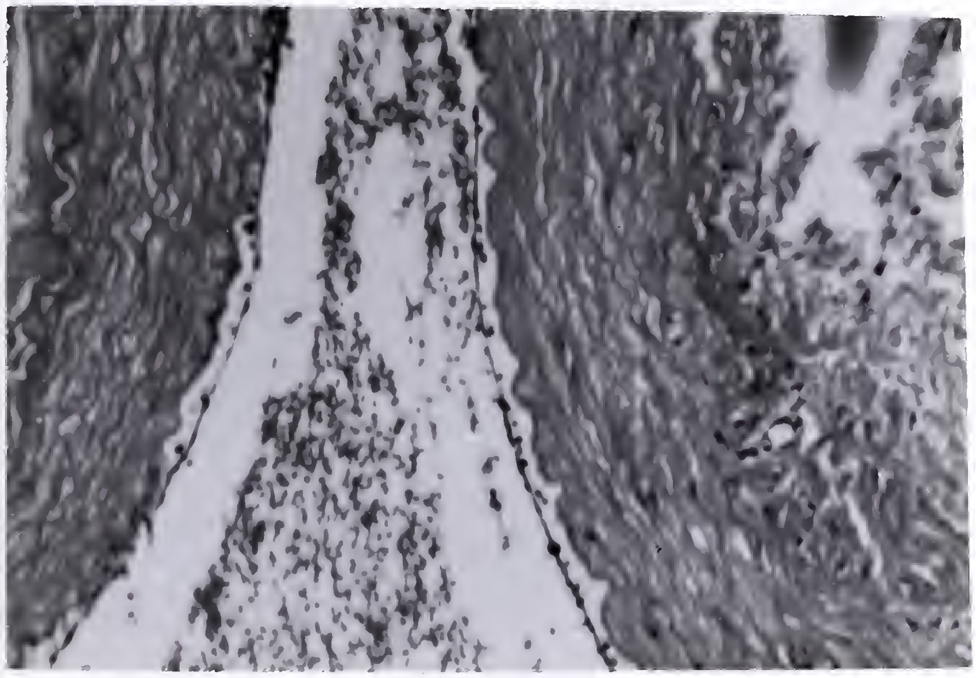


Figure 6. WA-44 aorta. Subintimal foam cellular accumulation, medial necrosis, adventitial hemorrhage. (H & E, 100x original magnification).

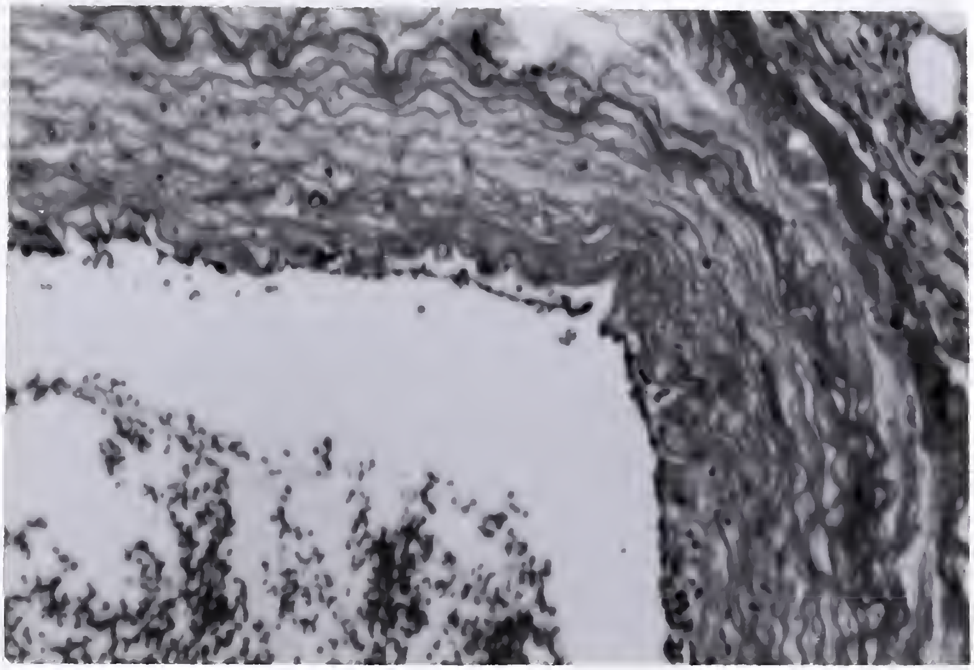


Figure 7. WA-44 aorta. Subintimal proliferation, medial necrosis, adventitial reaction with polys and mononuclear cells. (H & E, 100x original magnification).

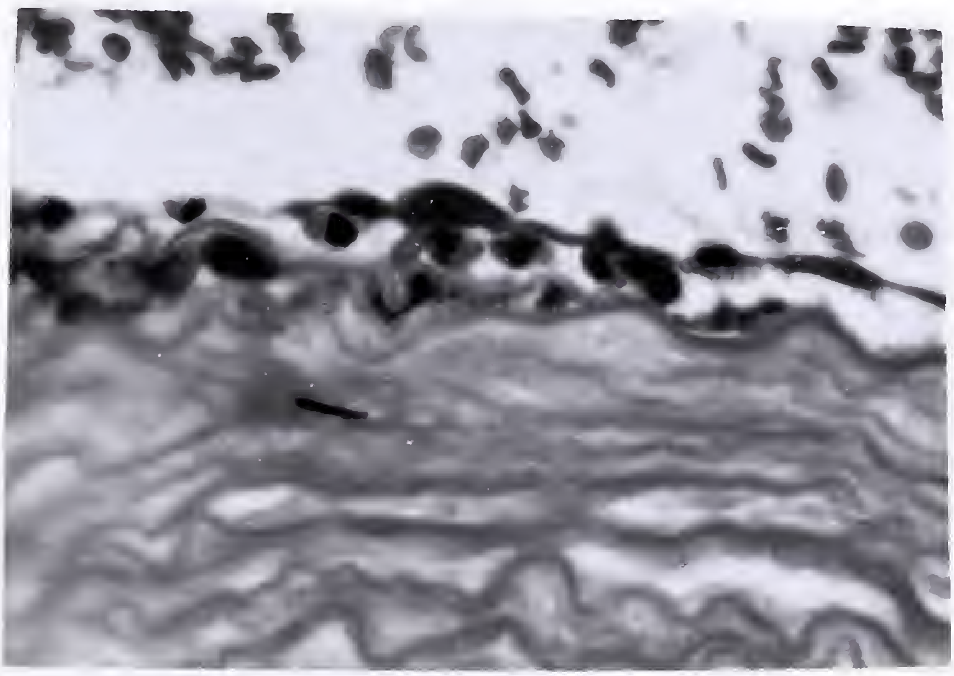


Figure 8. WA-44 aorta. Subendothelial space with many foam cells. (H & E, 450x original magnification).

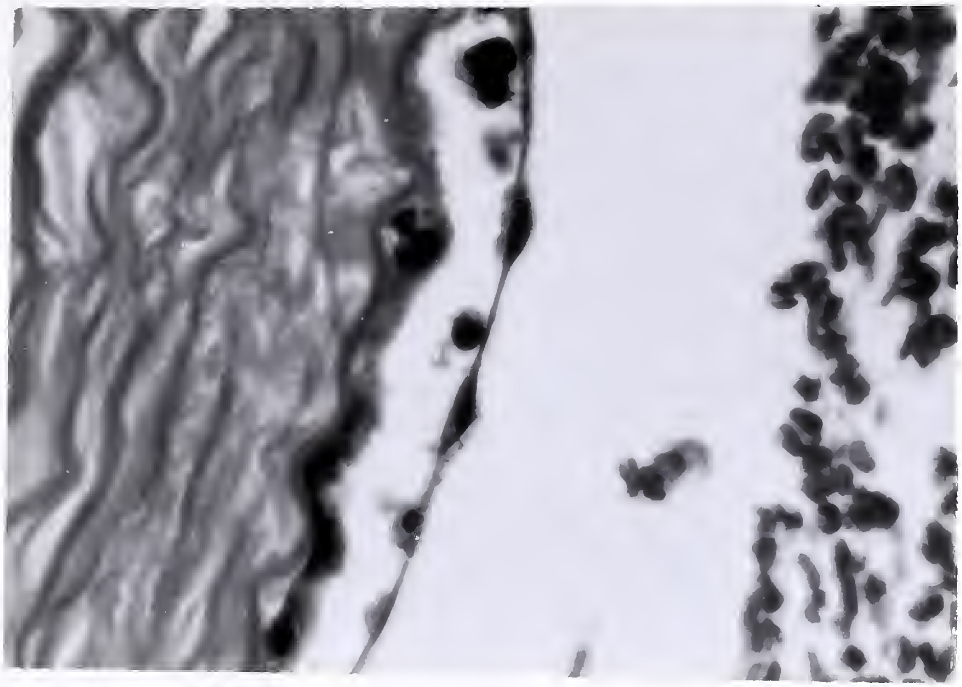


Figure 9. WA-44 aorta. Monocytes and young foam cells in subendothelial space.
(H & E, 450x original magnification).

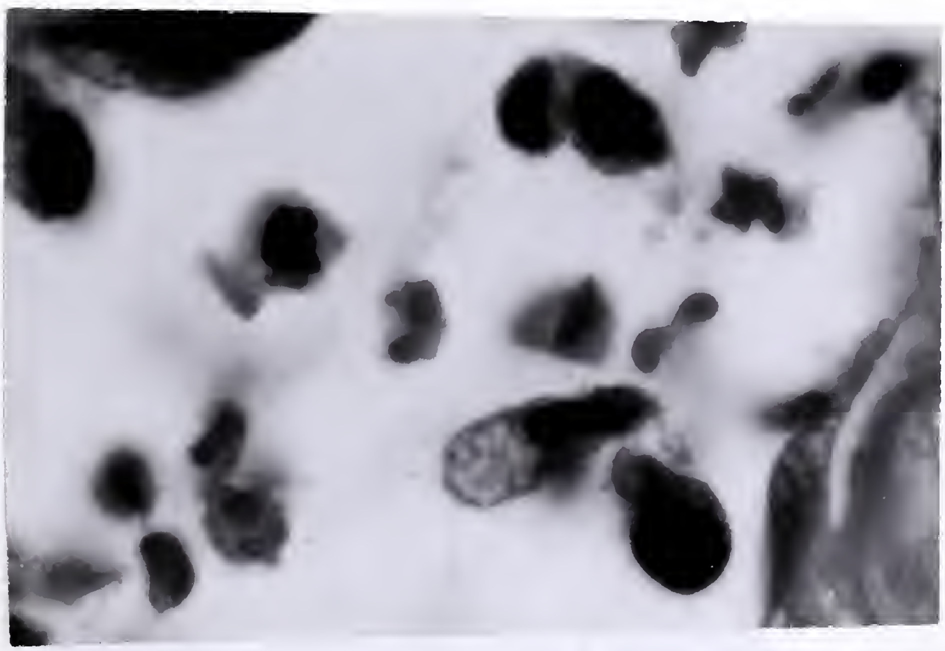


Figure 10. WA-44 aorta. "Tadpole" cell (young foam cell) in aortic lumen.
(H & E, 1000x original magnification).

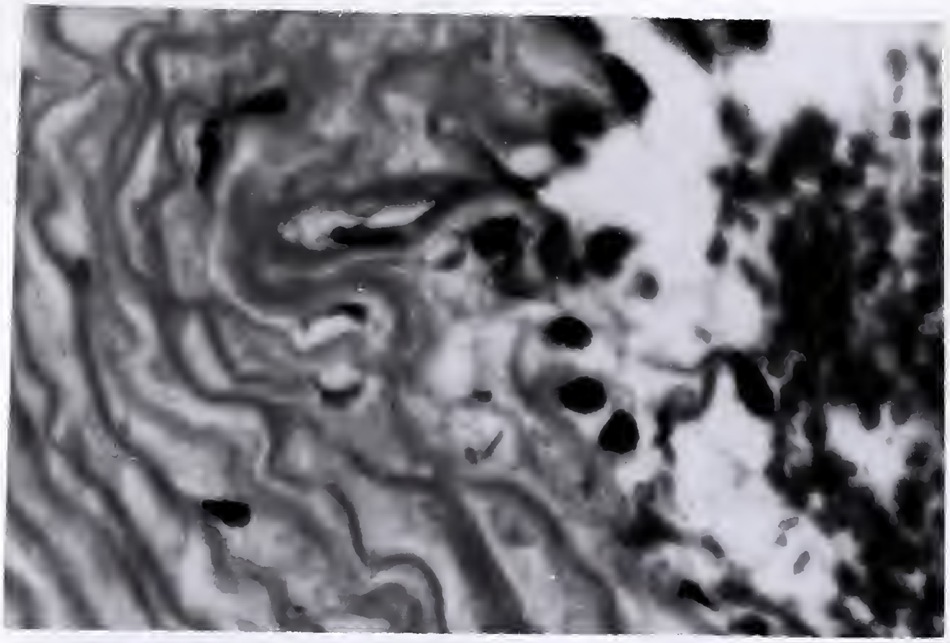


Figure 11. WA-44 aorta. Intimal foam cells, "tadpole" cell in lumen. H & E, 450x original magnification).

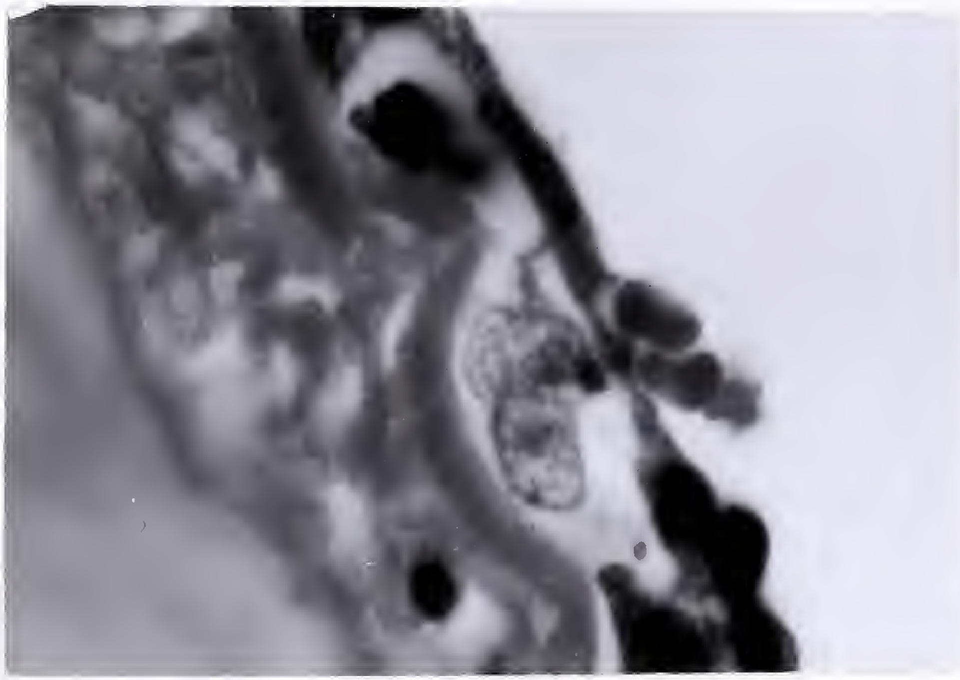


Figure 12. WA-44 aorta. Foam cell within subendothelial space. (H & E, 1000x original magnification).

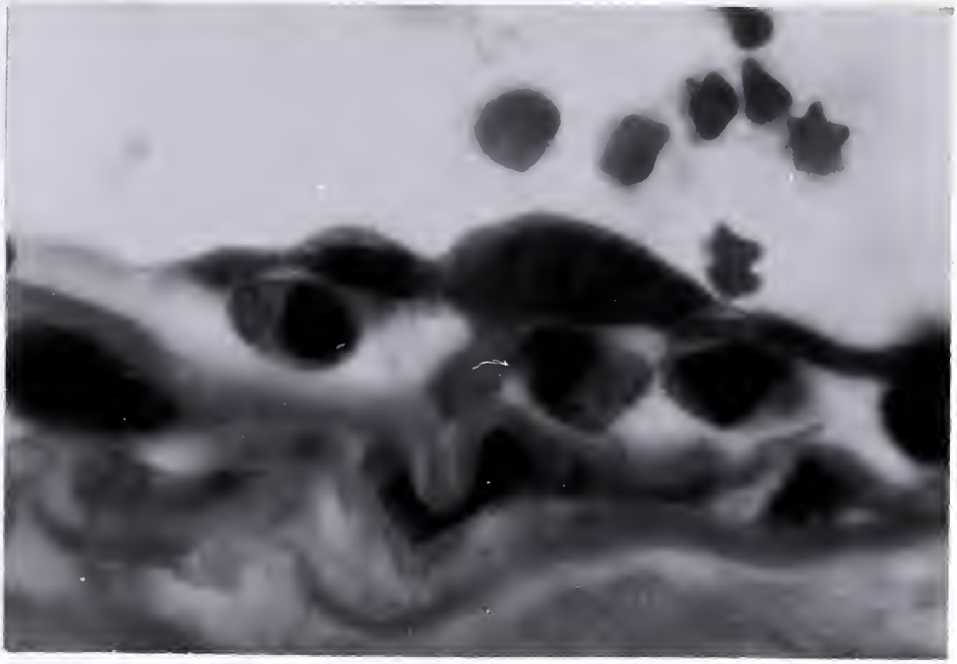


Figure 13. WA-44 aorta. Subendothelial foam cells, multinucleated endothelial cell.
(H & E, 1000x original magnification).

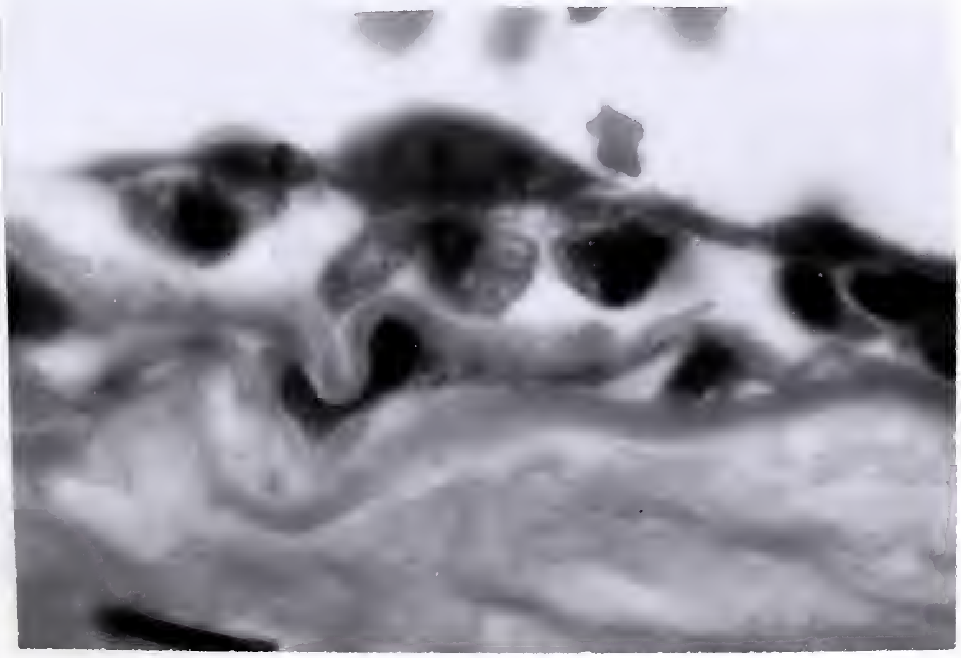


Figure 14. WA-44 aorta. Same as Figure 13 but different exposure.
(H & E, 1000x original magnification).

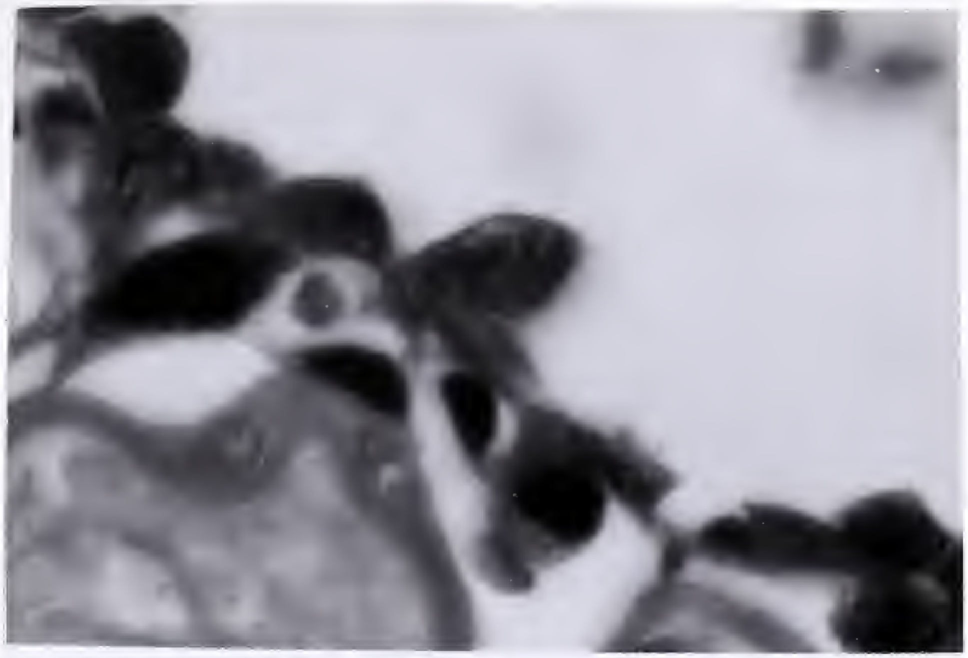


Figure 15. WA-44 aorta. Lipid-laden magrophage entering subendothelial space.
(H & E, 1000x original magnification).

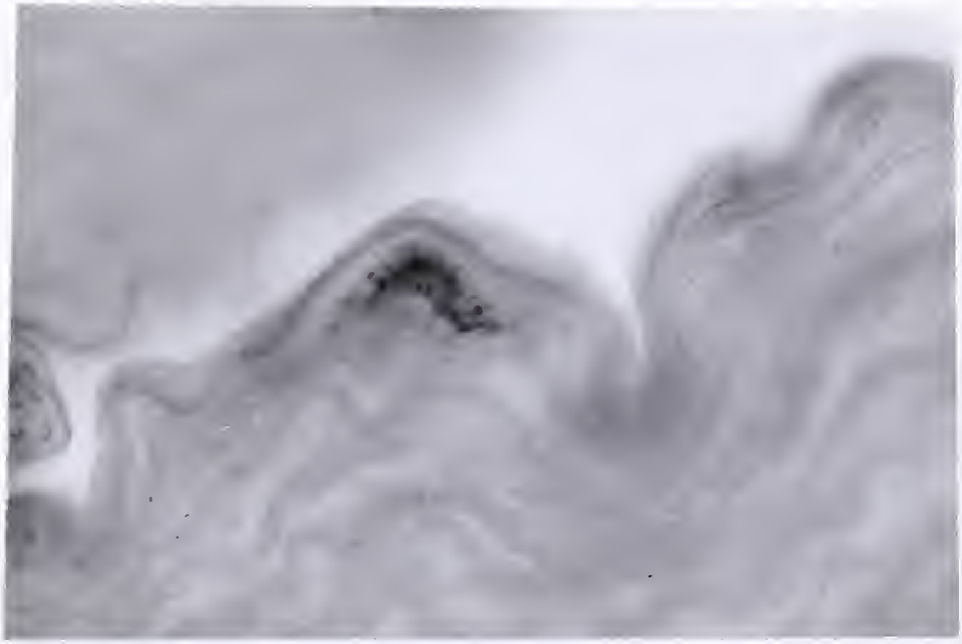


Figure 16. WA-44 aorta. Lipid droplets within foam cell beneath internal elastic lamina.
(Sudan IV, frozen section. 150x original magnification).

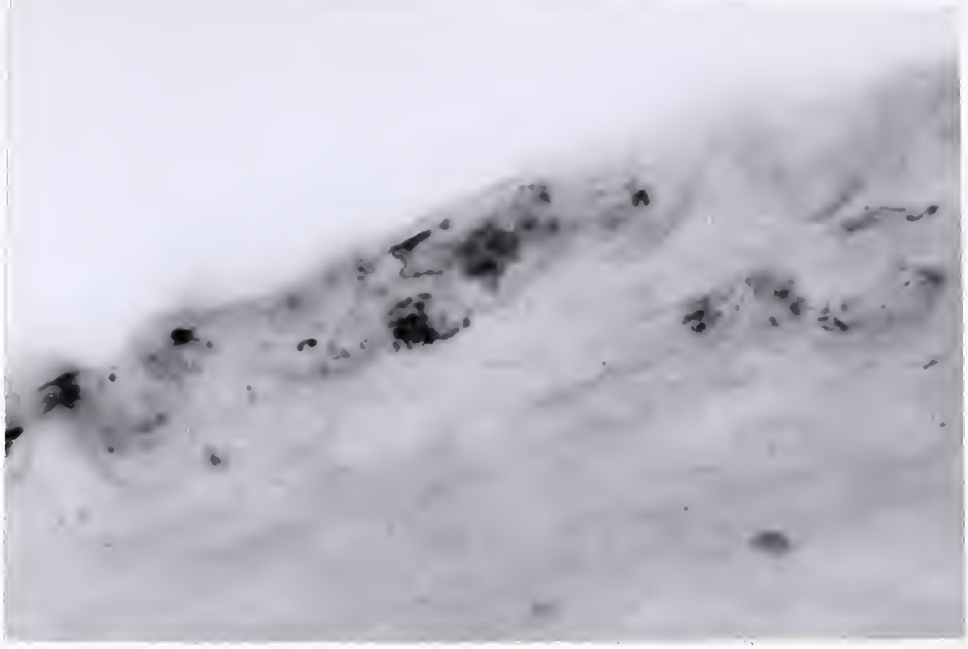


Figure 17. WA-44 aorta. Foam cells with lipid droplets within intima. (Sudan IV, frozen section. 450x original magnification).

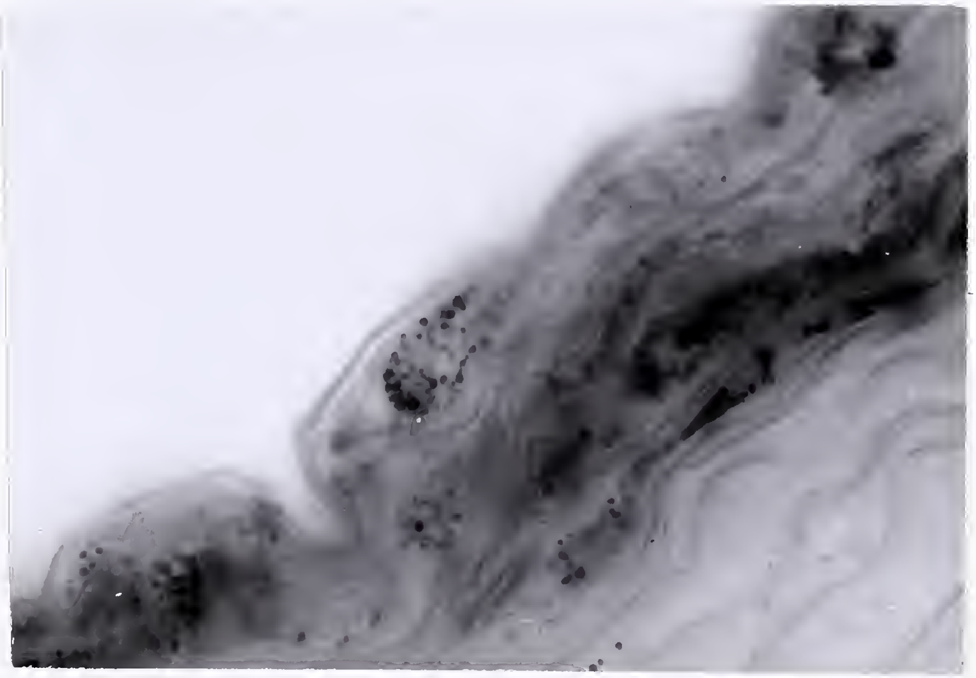


Figure 18. WA-44 aorta. Foam cellular and "free" lipid deep within intima.
(Sudan IV, frozen section, 450x original magnification).

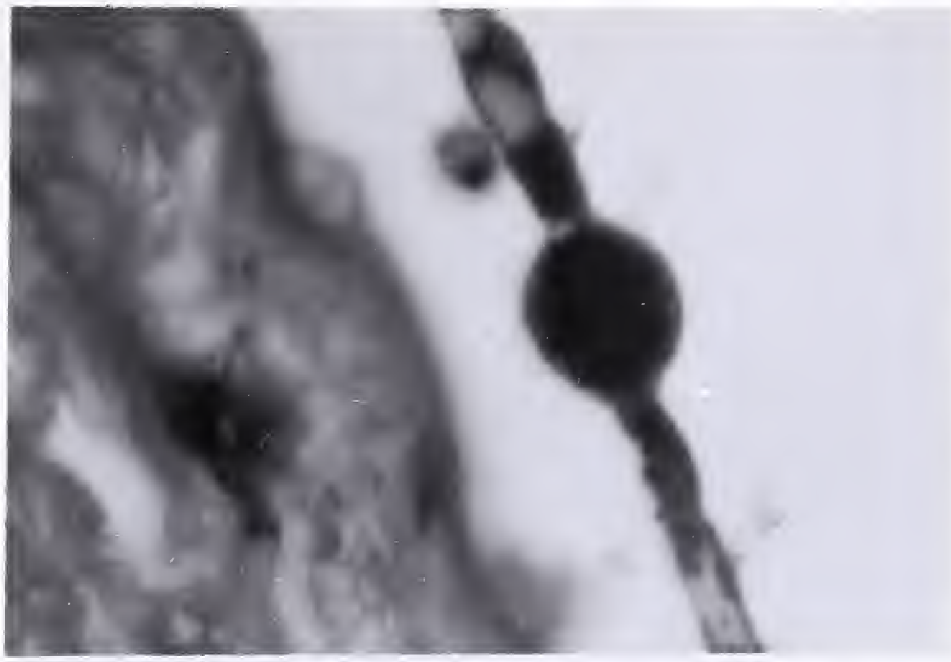


Figure 19. WA-44 aorta. Endothelial cell in mitosis and young sub-endothelial foam cells.
(H & E, 1000x original magnification).

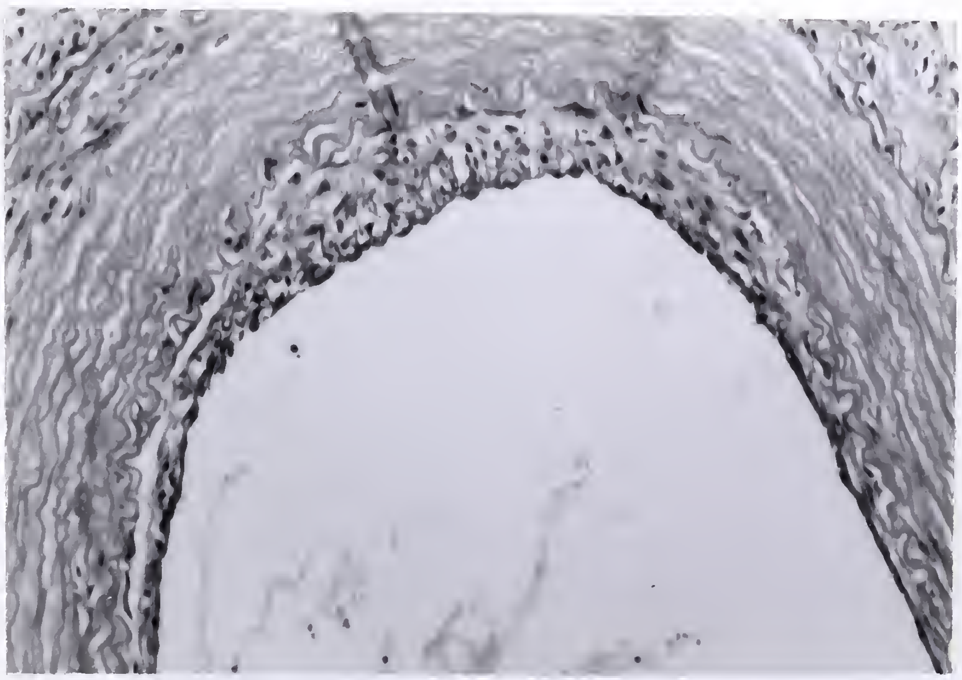


Figure 20. WA-60 aorta thirteen days after intravenous saline and eleven days after freezing. Marked subintimal cellularity and proliferation.
(H & E, 100x original magnification).

Figure 21. WA-59 or 60 aorta. Intimal proliferation with little visible lipid in aortic wall.
(Sudan IV, frozen section, 100x original magnification).

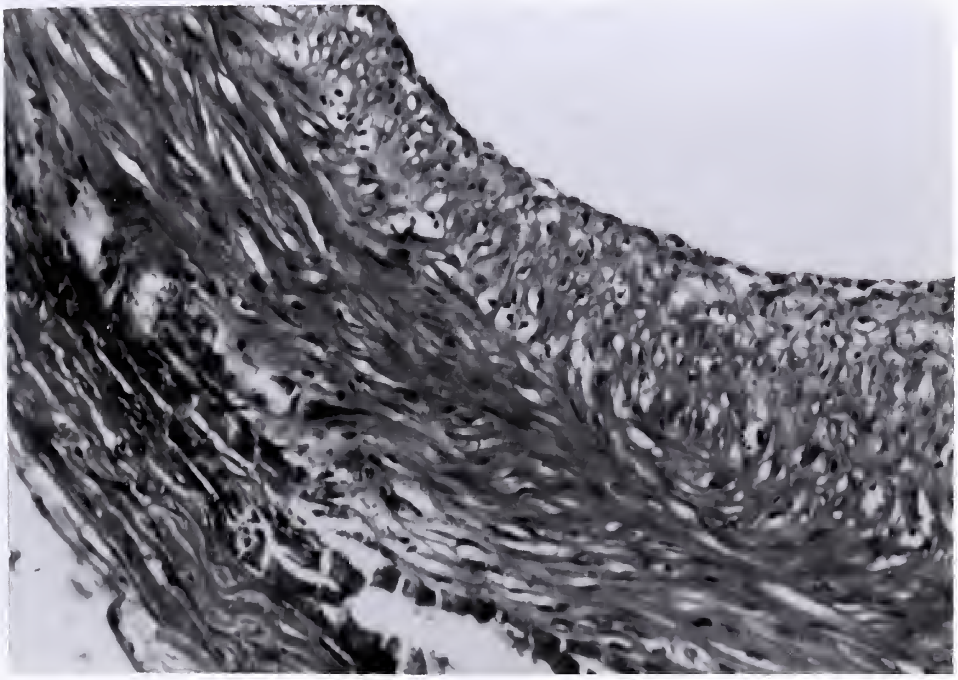


Figure 22. WA-49 aorta twenty-four days after Triton and twenty days after freezing. Proliferated intimal region, medial necrosis with calcification.
(H & E, 100x original magnification).

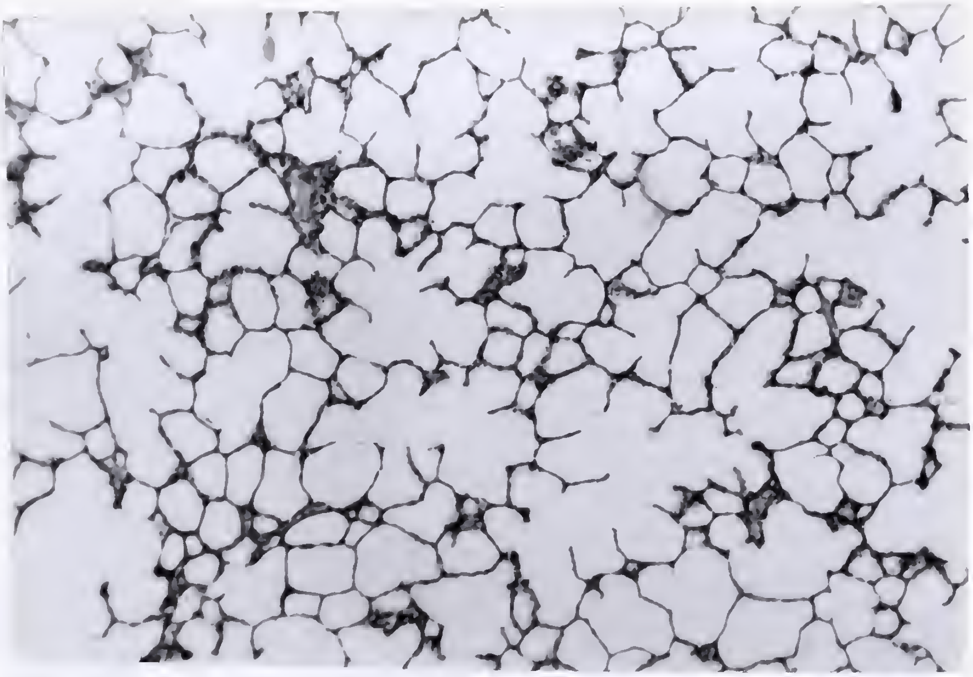


Figure 23. WA-60 lung demonstrates no pneumonia.
(H & E, 40 x original magnification).

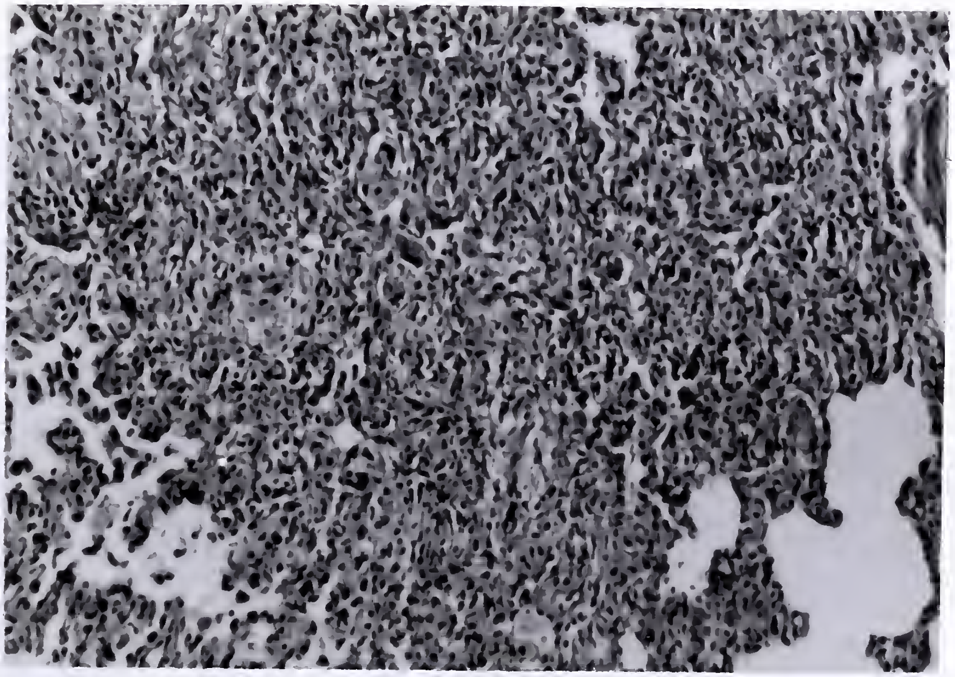


Figure 24. WA-49 lung. Lipoid pneumonia with mononuclear cells, giant cells, hemorrhage and foam cells.
(H & E, 100x original magnification).

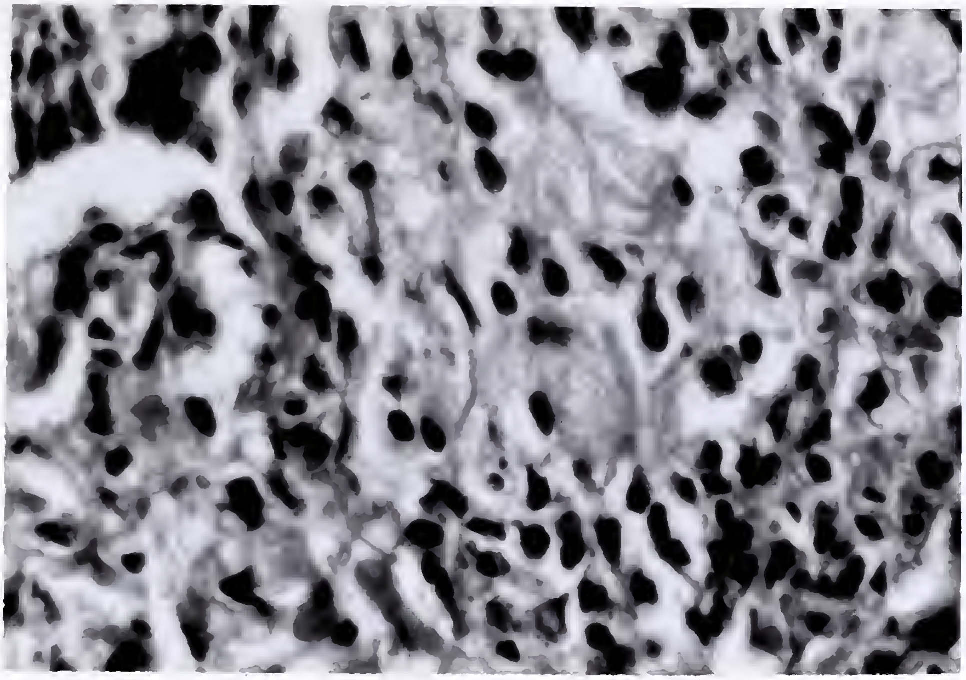


Figure 25. WA-49 lung. Lipoid pneumonia with foamy lipophages. (H & E, 450x original magnification).

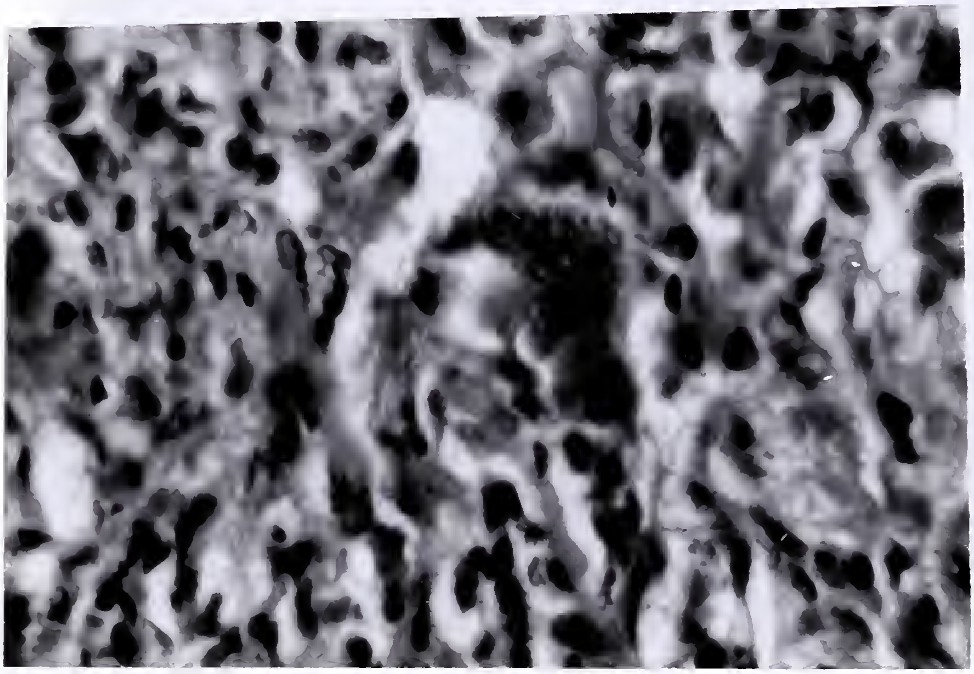


Figure 26. WA-49 lung. Lipoid pneumonia with giant cell. (H & E, 450x original magnification).

Figure 27. WA-50 aorta thirty-two days after Triton and twenty-eight days after aortic freezing. Subintimal proliferation and slight stainable lipid.
(Sudan IV, frozen section, 100x original magnification).

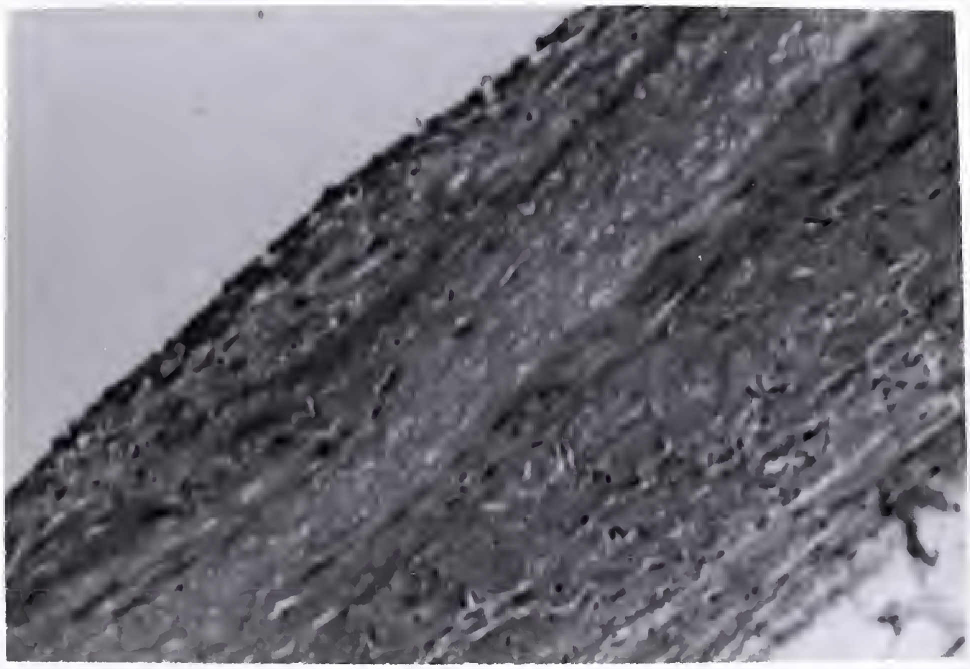


Figure 28. WA-50 aorta. Extensive medial necrosis.
(H & E, 40x original magnification).

Figure 29. WA-50 liver. Small amount of lipid still present thirty-two days after single Triton injection.
(Sudan IV, frozen section, 40x original magnification).

Chart 1

Summary of Preliminary Experiments with Pressors

| Rabbit | Pressor Used | Wt. (lb.) | Sacrifice | Results |
|---|---|---|---|---|
| WA-1 | epinephrine 1 cc. 1:10000 10-1 cc. injections/15 min. | ~ 6 | *20 min. | (mydriasis, cyanotic, weak. died 5 min. after final inject.) Pulmonary congestion and edema. abdominal wall musc. petechiae. |
| WA-2 | epinephrine 1:10000 4-1 cc. injections/15 min. | ~6 | *2-4 hrs. | died 2-4 hrs. after epinephrine. |
| WA-3 | epinephrine 1:10000 3-1 cc. injections/90 min. | 6 | *12 hrs. | renal tubular edema. |
| WA-4 | epinephrine 1:10000 2-1 cc., then 1-2 cc. injection q. 24 hrs. | ~ 6 | 5th. day | fibrin clot right pleural cavity. left ventricular aneurysm. pulmonary congestion. |
| WA 5 | epinephrine 1:10000 1-2 cc. injection. | 4.7 | 54 hrs. | petechial pulmonary hemorrhages |
| WA-6 | Levophed(R) (norepinephrine) 1 cc. 1/5 strength | 7.4 | 45 hrs. | double left renal artery. lungs clear pulmonary effusion. |
| WA-7 | Levophed 2 cc., then 5 cc. 1/5 strength at 48, 96 hrs. | 6.5 | 1 week | rapid induction tolerance. congested liver, lungs, kidneys. sacrificed week after <u>first</u> Levophed. |
| WA-8 | Tyramine-HCl 7.5 cc. of 1% over 2 min./ repeat q. 24 hrs. for 6 days. | 5 | 8th. day | perivascular edema in lungs, heart. sacrificed 8 days after <u>first</u> Levophed. |
| WA-9 | Tyramine-HCl 7.5 cc. of 1% over 1 min. | 8.2 | *3 min. | 5x7 mm. accessory spleen. tachycardia $\longrightarrow$ cardiac arrest, seizures $\longrightarrow$ death. |

* Did not survive length of experiment.

Chart 2

Summary of Experiments Using Pituitary Extract and/or Horse Serum

Key to types of pituitary extract used:
1-whole dried beef pituitary extracts/10 cc. sterile saline.
2-AS#1, but filtered with ether.
3-AS#1, but boiled in ethanol/1 hour.
4-Defatted beef pituitary extract in saline.

| Rabbit | Pituitary Extract X type (mg) | Days Given | Horse Serum (cc. and # of times given) | Days Given | Findings |
|---|---|---|---|---|---|
| WA-10 | 130 mg.[1] | 22 | 25 cc. x 2 | 1,20 | No lipemia. |
| WA-11 | | | 25 cc. | 1 | Found dead overnight. |
| WA-12 | 130 mg.[1] | 22 | 25 cc. x 2 | 1,20 | No lipemia. |
| WA-13 | 100 mg.[4] | 1 | | | Found dead overnight. |
| WA-14 | 100 mg.[1], 420 mg.[3] | 1,20 | | | Serum samples at 24, 52, 100 hours. |
| WA-15 | 200 mg.[4], 600 mg.[2] | 1,20 | | | Serum samples milky at 24, 52, 100 hours. |
| WA-16 | 200 mg.[1] | 1 | | | Serum sample at 24 hours, then died. |
| WA-17 | 2000 mg.[1] | 1 | | | Found dead next day, autolysed. |
| WA-18 | 1000 mg.[1] | 1 | | | Found dead next day, autolysed. |
| WA-19 | 1000 mg.[1] | 1 | | | Died next day after blood drawn; lipemia, fatty liver. |
| WA-20 | 1000 mg.[1] | 1 | | | Serum at 24 hours lipemia; died one hour later; pulmonary edema. |

| Rabbit | Pituitary Extract X type (mg) | Days Given | Horse Serum (cc. and # of times given) | Days Given | Findings |
|---|---|---|---|---|---|
| WA-21 | | | 20 cc. x 3 | 1,7,14 | Died 4 minutes after third serum; anaphylaxis. |
| WA-22 | 600 mg.[2] x 2 | 17,21 | 20 cc. x 3 | 1,7,14 | Serum not ipemic. |
| WA-23 | 600 mg.[2] x 2 | 17,21 | 20 cc. x 3 | 1,17, 14 | |
| WA-24 | 600 mg.[2] x 2 | 17,21 | 20 cc. x 2 then 12 cc. | 1,17, 14 | Died with broken back. |
| WA-25 | 750 mg.[1] | 1 | | | Diarrhea 45 min. after pituitary; found dead next AM. |
| WA-26 | 500 mg.[1] | 1 | | | Died in 48 hours; autolysed; left renal cortical scar. |
| WA-27 | 750 mg.[1] | 1 | | | Found dead next AM with abdominal hemorrhage. |
| WA-28 | 1000 mg.[3] | 1 | | | Died day 2 while being bled. |
| WA-29 | 375 mg.[1] | | | | No gross lipemia. |
| WA-30 | 200 mg.[2], 400 mg.[2], 600 mg.[2], 500 mg.[2] | 1,3, 9,10 | | | No gross lipemia. |
| WA-31 | 200 mg.[2], 400 mg.[2], 600 mg.[2], 500 mg.[2], 1000 mg.[2] | 1,3 4,5 18 | | | |

| Rabbit | Pituitary Extract X type (mg) | Days Given | Horse Serum (cc. and # of times given) | Days Given | Findings |
|---|---|---|---|---|---|
| WA-32 | 200 mg.$^2$, 400 mg.$^2$, 600 mg.$^2$, 650 mg.$^2$ | 1,3,9, 10 | | | |
| WA-33 | 350 mg.$^2$, 400 mg.$^2$ | 1,3 | | | Sacrificed day 4 |
| WA-34 | 700 mg.$^2$ | 14 | 20 cc. x 3 | 1,7, 13 | Serum slightly lipemic on day 15. |
| WA-35 | | | 20 cc. x 3 | 1,7, 13 | Diarrhea day 8; died day 13; anaphylaxis and congested liver. |
| WA-36 | 700 mg.$^2$ | 15 | 20 cc. x 3 | 1,8, 14 | |
| WA-37 | 700 mg.$^2$ | 15 | 20 cc. x 3 | 1,8, 14 | Serum milky on day 16. |
| WA-38 | 600 mg.$^2$, 500 mg.$^2$ | 1,2 | | | Diarrhea AM day 2 |
| WA-39 | 650 mg.$^2$ | 25 | 33 cc. x 3 | 1,14, 21 | One pulmonary arteriole with fibrinoid necrosis; pericarditis; liver not fatty; diffuse plural effusion; normal aortas and kidneys. |
| WA-40 | | | 20 cc. x 1 | 1* | |
| WA-41 | 650 mg.$^2$ | 25 | 33 cc. x 3 | 1,14, 21 | |
| WA-42 | 650 mg.$^2$ | 25 | 33 cc. x 3 | 1,14 21 | |

* Stolen from animal room day 2.

Chart 3

Baseline Serum Lipid Values of Rabbits

| Rabbit | Weight (lbs.) | TTL Chol. mg.% | Free Chol. mg.% | Fatty Acids meq./. | Phos-L. mg.% | Triglyc. meq./1 |
|---|---|---|---|---|---|---|
| WA-13 | 5.4 | 60.6 | 33.0 | 15.0 | 6.9 | 11.3 |
| 14 | 4.8 | 81.0 | 48.0 | 15.0 | 6.0 | 10.6 |
| 15 | 5.25 | 60.6 | 19.0 | 15.0 | 6.1 | 10.4 |
| 16 | 5.0 | 72.0 | 33.0 | 17.0 | 7.0 | 12.9 |
| 17 | Died | | | | | |
| 18 | 5.0 | 74.5 | 21.0 | 12.0 | 5.5 | 7.4 |
| 19 | 7.0 | 46.0 | 20.0 | 12.6 | 4.1 | 9.5 |
| 20 | 7.0 | 72.5 | 43.0 | 12.0 | 4.8 | 8.5 |
| 21 | 4.75 | Died | | | | |
| 22 | 4.75 | 65.1 | 13.0 | 13.6 | 5.1 | 9.3 |
| 23 | 4.75 | 92.1 | 13.0 | 10.4 | 4.3 | 6.8 |
| 24 | 5.0 | 67.1 | 9.1 | 8.0 | 3.8 | 4.3 |
| 25 | 5.0 | Died | | | | |
| 26 | 5.0 | 128.5 | — | 14.4 | 5.8 | 8.6 |
| 27 | 7.0 | Died | | | | |
| 28 | 5.0 | 74.3 | — | 11.2 | 5.6 | 6.6 |
| 29 | 5.0 | 39.4 | 9.1 | 11.2 | 4.0 | 8.1 |
| 30 | 7.0 | 47.0 | 13.9 | 12.0 | 4.3 | 8.7 |
| 31 | 7.0 | 44.0 | 11.7 | 8.0 | 3.8 | 5.0 |
| 32 | 7.0 | 77.5 | 17.0 | 11.2 | 5.6 | 6.4 |
| 33 | 7.0 | 39.0 | 27.0 | 7.2 | 2.8 | 5.3 |
| 38 | 4.5 | 65.5 | 10.2 | 15.7 | 6.7 | 9.9 |
| 43 | 8.9 | 36.4 | — | 6.8 | 3.0 | 4.4 |
| 44 | 8.25 | 47.6 | — | 8.0 | 5.2 | 4.1 |
| 45 | 8.4 | 70.2 | — | 18.0 | 6.0 | 13.2 |
| 46 | 10.4 | 23.0 | — | 6.0 | 2.5 | 4.1 |
| 47 | 7.9 | 47.6 | — | 8.0 | 3.3 | 5.2 |
| 48 | 9.4 | 24.4 | — | 18.0 | 4.4 | 15.0 |
| 49 | 8.5 | 54.0 | — | 8.0 | 5.8 | 3.6 |
| 50 | 9.3 | 29.8 | — | 4.0 | 2.5 | 1.9 |
| 51 | 8.5 | 74.4 | — | 12.0 | 6.4 | 6.9 |
| 53 | 8.5 | 106.8 | — | 10.7 | 8.6 | 3.7 |
| 54 | 8.5 | 72.4 | — | 9.7 | 6.0 | 4.9 |
| 55 | 7.9 | 60.6 | — | 8.3 | 5.6 | 3.9 |
| 56 | 8.4 | 108.2 | — | 8.3 | 5.5 | 3.1 |
| 59 | 6.1 | 148.4 | — | 18.0 | 9.0 | 10.0 |
| 60 | 9.25 | 161.0 | — | 14.5 | 8.5 | 1.2 |
| 67 | — | 67.0 | — | 9.5 | 5.5 | 5.1 |
| 68 | — | 83.0 | — | 9.5 | 5.0 | 3.1 |
| 69 | — | 95.0 | — | 9.5 | 6.0 | 4.2 |
| 70 | — | 59.6 | — | 10.2 | 3.1 | 6.3 |
| 71 | — | 43.0 | — | 5.0 | 4.0 | 1.9 |
| 72 | — | 85.4 | — | 7.5 | 6.0 | 2.4 |

Chart 4

A Comparison of Fatty Acid Determinations on Baseline Samples

| Rabbit | Fatty Acids meq./l Stoddard & Drury | Fatty Acids meq./. Stern and Shapiro |
|---|---|---|
| 53B | 10.0 | 11.3 |
| 54B | 9.3 | 10.0 |
| 43-9 days | 13.0 | 9.5 |
| 55B | 8.6 | 8.0 |
| 56B | 8.6 | 8.0 |
| 49#3 | 7.3 | 8.7 |
| 53#3 | 40.0 | 40.5 |
| 55#3 | 18.0 | 15.5 |
| 56#3 | 52.6 | 54.5 |

Chart 5

Triton-Chemistries

| Rabbit | Time | Cholesterol mg.% Total | Free | Fatty Acids meq./l. | Phospho-lipids mg.% | Trigly-cerides meq./l. |
|---|---|---|---|---|---|---|
| WA-43 | Base | 36.4 | | 6.8 | 3.0 | 4.4 |
| | 5½ hr | 118.1 | 34.3 | 32.5 | 8.5 | 25.4 |
| | 23 hr | 330.0 | 182.0 | 90.0 | 25.0 | 71.7 |
| | 9 days | 145.0 | 41.5 | 11.8* | 7.8 | |
| WA-44 | Base | 47.6 | | 8.0 | 5.2 | 4.1 |
| | 23½ hr | 366.6 | 149.0 | 150.0 | 37.5 | 122.6 |
| | 120 hr | 507.9 | 350.0 | 125.0 | 45.0 | 95.1 |
| WA-45 | Base | 70.2 | | 18.0 | 6.0 | 13.2 |
| | 23½ hr | 461.6 | 270.0 | 180.0 | 41.3 | 151.2 |
| WA-46 | Base | 23.0 | | 6.0 | 2.5 | 4.1 |
| | 17 hr | 238.0 | 68.0 | 110.0 | 22.5 | 92.6 |
| WA-47 | Base | 47.6 | | 8.0 | 3.3 | 5.2 |
| | 17 hr | 226.6 | 103.0 | 75.0 | 20.0 | 60.2 |
| | 48 hr | 482.2 | 322.0 | 250.0 | 43.5 | 221.4 |
| WA-48 | Base | 24.4 | | 18.0 | 4.4 | 15.0 |
| | 17 hr | 225.0 | 113.8 | 142.5 | 23.8 | 125.8 |
| | 48 hr | 456.5 | 351.0 | 210.0 | 42.5 | 182.4 |
| WA-49 | Base | 54.0 | | 8.0 | 5.8 | 3.6 |
| | 70 hr | 594.3 | 491.0 | 205.0 | 47.5 | 174.4 |
| | 19 days | | | | | |
| WA-50 | Base | 29.8 | | 4.0 | 2.5 | 1.9 |
| | 70 hr | 556.0 | 299.0 | | 53.2 | |
| | 27 days | | | | | |
| WA-51 | Base | 74.8 | | 12.0 | 6.4 | 6.9 |
| | 70 hr | 443.0 | 385.0 | 165.0 | 43.5 | 138.1 |
| WA-53 | Base | 106.8 | | 10.7* | 8.6 | |
| | 18 hr | 280.0 | 170.0 | 107.0 | 28.0 | |
| | 12 days | | | 40.2* | | ?#3 |
| WA-54 | Base | 72.4 | | 9.7 | 6.0 | |
| | 18 hr | 220.0 | 102.5 | 95.5 | 24.0 | |
| WA-55 | Base | 60.6 | | 8.3 | 5.6 | |
| | 20 hr | 150.0 | 106.0 | 55.0 | 17.5 | ?#3 |
| | 7 days | | | 16.8 | | |

| Rabbit | Time | Cholesterol mg.% Total | Free | Fatty Acids meq./l. | Phospholipids mg.% | Triglycerides meq./l. |
|---|---|---|---|---|---|---|
| WA-56 | Base | 108.2 | | 8.3 | 5.5 | |
| | 20 hr | 247.5 | 167.4 | 84.0 | 21.5 | ?#3 |
| | 7 days | | | 53.5 | | |
| FOR CORNEAL INJECTION | | | | | | |
| WA-57 | | 224.1 | 120.0 | | 8.3 | 5.5 |
| WA-58 | | 269.8 | 175.5 | 10.0 | 2.5 | 6.1 |
| Supernate x 0.1 | | 70.0 | 34.1 | 15.0 | 8.8 | 8.9 |
| Subnate | | 168.0 | 127.5 | 35.0 | 20.0 | 22.3 |
| WA-59 | Base | 148.4 | | 18.0 | 9.0 | 10.0 |
| (Saline Control) | 42 hr | 160.7 | 34.1 | 12.6 | 11.0 | 3.0 |
| Freezing | 13 days | 63.5 | 12.4 | 12.0 | 5.0 | 8.3 |
| WA-60 | Base | 161.0 | | 14.5 | 8.5 | 1.2 |
| (Saline Control) | 42 hr | 127.5 | 37.8 | 17.6 | 15.0 | 6.6 |
| Freezing | 13 days | 113.5 | 13.0 | 12.5 | 6.0 | 5.8 |
| WA-63 | 95 hr | 475.0 | 438.0 | 210.0 | 46.0 | 182.4 |
| WA-64 | 95 hr | 745.0 | 337.0 | 110.0 | 29.0 | 92.0 |
| WA-67 | Base | 67.0 | | 9.5 | 5.5 | 5.1 |
| | 1 hr/ 20 min. | 57.5 | 24.6 | 10.6 | 3.0 | 7.7 |
| | 12 hr/ 40 min. | 136.1 | 88.0 | 46.7 | 12.0 | 38.4 |
| | 30 hr | 301.0 | 245.5 | 102.5 | 24.5 | 86.8 |
| | 29 hr/ 40 min. | | | | | |
| WA-68 | Base | 83.0 | | 9.5 | 5.0 | 3.1 |
| | 1 hr | 65.01 | 24.6 | 9.8 | 4.5 | 6.0 |
| | 12 hr/ 35 min. | 136.1 | 99.0 | 57.5 | 14.0 | 48.4 |
| | 30 hr | 305.4 | 215.0 | 127.5 | 23.0 | 111.8 |
| | 29½ hr | | | | | |
| | 7½ days | 350.2 | 137.5 | 27.5 | 17.5 | 11.5 |
| | 13 days | 200.0 | 56.2 | 21.0 | 9.0 | 12.1 |

| Rabbit | Time | Cholesterol mg.% Total | Free | Fatty Acids meq./l. | Phospholipids mg.% | Triglycerides meq./l. |
|---|---|---|---|---|---|---|
| WA-69 | Base | 95.0 | | 9.5 | 6.0 | 4.2 |
| | 1 hr/ 40 min. | 79.0 | 34.0 | 10.5 | 5.0 | 6.1 |
| | 12 hr/ 25 min. | 162.5 | 131.0 | 97.5 | 16.4 | 86.1 |
| | 78 hr | 367.3 | 234.4 | 125.0 | 22.5 | 107.3 |
| | 7½ days | 630.8 | 534.0 | 83.0 | 46.7 | 53.3 |
| | 13 days | 175.5 | 49.9 | 20.0 | 9.0 | 11.6 |
| WA-70 | Base | 59.6 | | 10.2 | 3.1 | 6.3 |
| | 1½ hr | 62.5 | 31.0 | 12.5 | 5.5 | 8.1 |
| | 12¼ hr | 124.5 | 95.0 | 50.0 | 13.1 | 41.7 |
| | 78 hr | 662.0 | 320.0 | 290.0 | 45.0 | 255.0 |
| | 7½ days | 726.3 | 526.3 | 115.0 | 60.0 | 77.0 |
| | 13 days | 205.0 | 41.5 | 20.0 | 11.0 | 9.5 |
| WA-71 | Base | 43.0 | | 5.0 | 4.0 | 1.9 |
| | 30 hr | 388.5 | 235.5 | 125.0 | 24.0 | 109.8 |
| | 76 hr | 602.5 | 266.4 | 205.0 | 44.0 | 160.6 |
| | 95 1/3 hr | 635.0 | 507.9 | 190.0 | 52.0 | 156.6 |
| | 7½ days | 496.3 | 299.0 | 60.0 | 40.0 | 31.7 |
| | 13 days | 56.0 | 13.0 | 15.0 | 5.0 | 11.5 |
| WA-72 | Base | 85.4 | | 7.5 | 6.0 | 2.4 |
| | 28 hr/ 5 min. | 388.4 | 306.0 | 155.5 | 29.0 | 136.6 |
| | 76¼ hr | 585.2 | 266.3 | 175.0 | 42.5 | 141.9 |
| | 95¼ hr | 680.0 | 594.3 | 118.0 | 44.0 | 90.3 |
| | 7½ days | 500.0 | 260.0 | 27.5 | 15.0 | 12.6 |
| | 13 days | 189.0 | 50.0 | 19.0 | 9.0 | 10.2 |

Chart 6

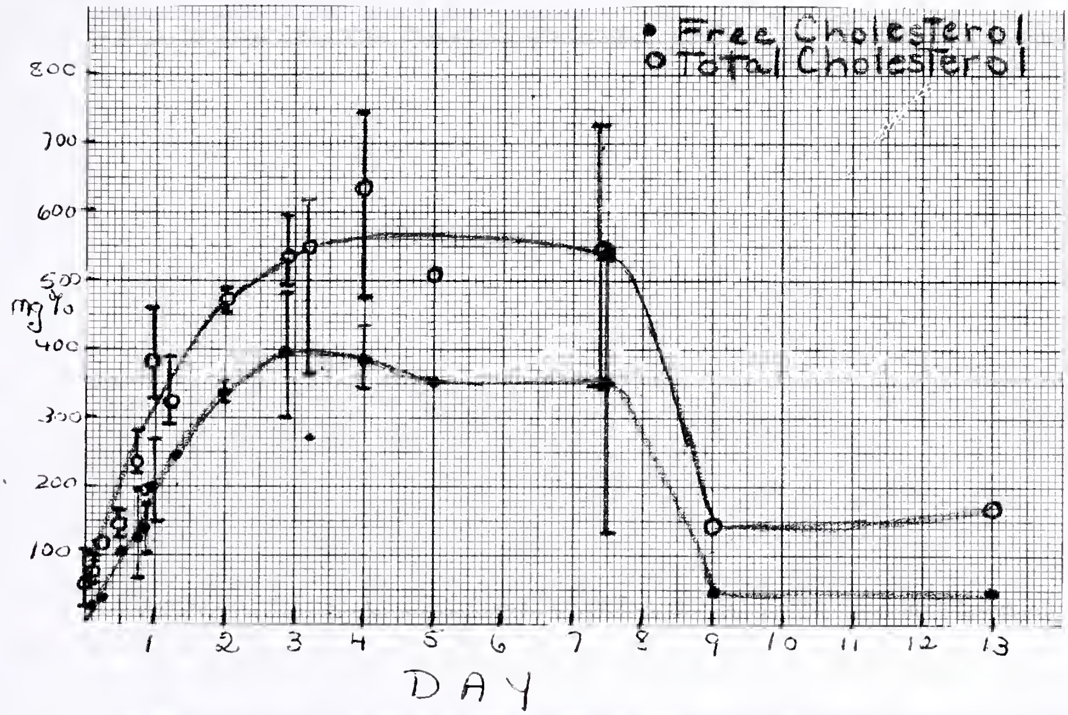



Chart 7

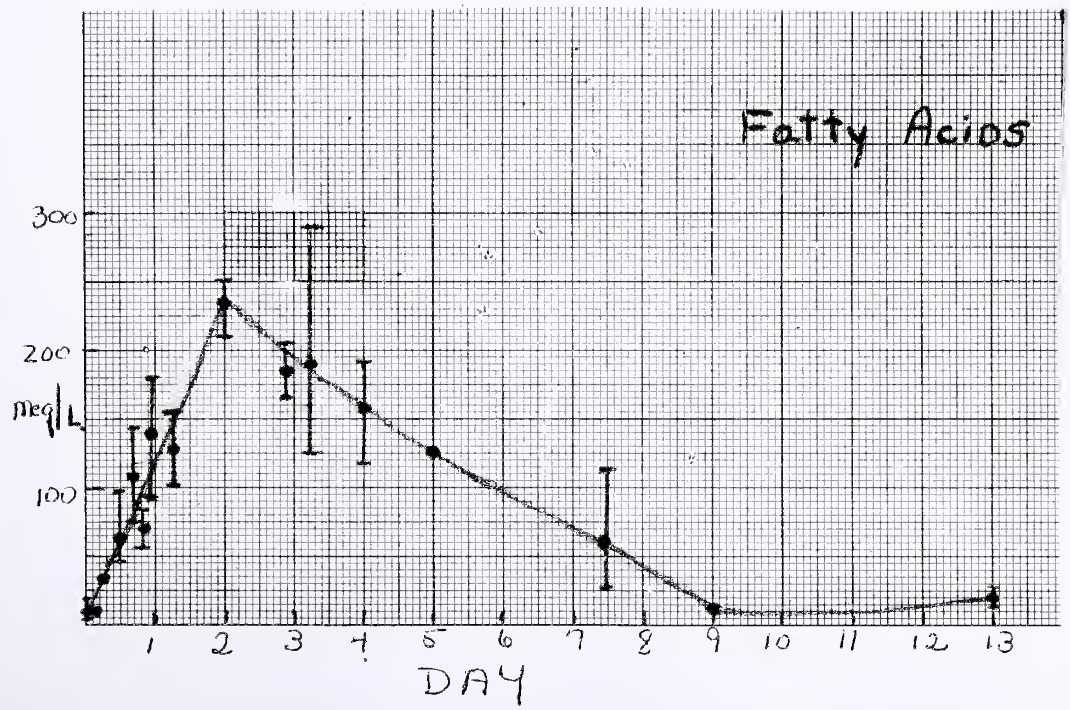


Chart 8

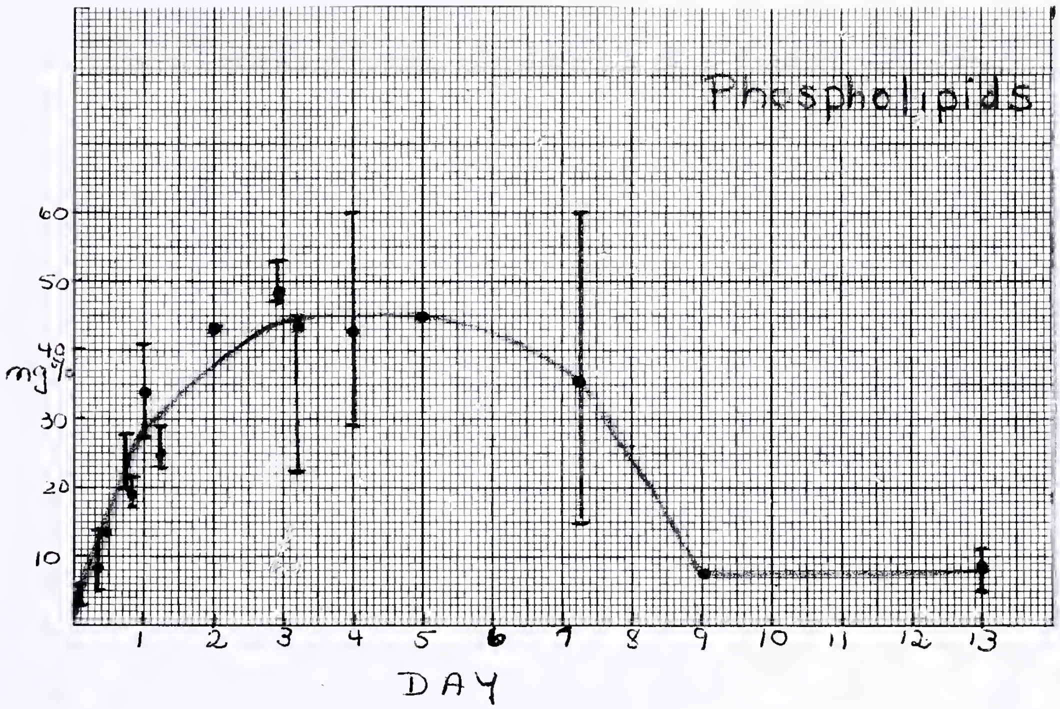


Chart 9

Rabbits Receiving Triton and/or Aortic Freezing

| Number Weight (lbs.) Sex | Amount (cc.) of Triton, | Day Given | Day of Aortic Freezing | Gross and Microscopic Observations | Serum Grossly Lipemic | Sacrificed Days $\bar{p}$ Triton | Days $\bar{p}$ Freezing |
|---|---|---|---|---|---|---|---|
| 43, 8.9 ♀ | 8.5 | 1 | 2 | Renal cortical scars. | yes | 9 | 8 |
| 44, 8.25 ♂ | 8.25 | 1 | 2 | Aorta - periadventitial hemorrhage, granulation and fat, medial necrosis, intimal proliferation, subintimal cellular accumulation of foam cells and monocytes.<br>Normal heart, lung, kidney. | yes | 5 | 4 |
| 45, 8.4 ♂ | 8.4 | 1 | 2 | Aorta - subendothelial foam cells with little stainable lipid; medial calcification, hemorrhage and necrosis with slightly more lipid. (accessory spleen).<br>Ventricular septal myocardial infarction; pulmonary arteriolar intimal proliferation. | yes | 3 mos. | 3 mos. |
| 46, 10.4 ♂ | 11.8 | 1 | — | Animal died with broken back, autolysed. Fatty liver; gross lipemia. | yes | 1 | — |
| 47, 7.9 ♂ | 9.0 | 1 | — | Congested liver without lipid; pulmonary arteriolar intimal proliferation; pulmonary hemorrhage.<br>One coronary arteriole with cells and adherent to subintimal membrane. | yes | 3 mos. | 3 mos. |

| Number Weight (lbs.) Sex | Amount (cc). of Triton, Day Given | Day of Aortic Freezing | Gross and Microscopic Observations | Serum Grossly Lipemic | Sacrificed Days $\bar{p}$ Triton | Days $\bar{p}$ Freezing |
|---|---|---|---|---|---|---|
| 48, 9.4 ♂ | 10.75 1 | — | Animal missing - never found. | yes | — | — |
| 49, 8.5 ♀ | 9.6 1 | 4 | Aorta - extensively calcified media; some subintimal lipid and cell accumulation.<br>Lipoid pneumonia with mononuclear cells, giant cells, hemorrhage and foam cells; pulmonary arteriolar intimal proliferation.<br>No renal tubular lipid; renal cortical scars.<br>Inferior vena caval phlebitis. | yes | 24 | 20 |
| 50, 9.3 ♂ | 11.6 1 | 4 | Aorta - subintimal proliferation; lipid deposit-ion in media; necrotic media.<br>heart - no stainable lipid.<br>lung - no pulmonary arteriolar lipid; some consolidation.<br>liver - virtually no fat | yes | 32 | 28 |
| 51, 8.5 ♀ | 9.6 1 | 4 | Aorta - slight subintimal cellularity without lipid.<br>heart - normal; normal amount of peritoneal fat.<br>lungs - normal; slight subintimal cell infiltration without lipid in pulmonary arterioles. | yes | 3 mos. | 3 mos. |
| 52 — — | —— — | — | Died of cardiac tamponade after withdrawl of first blood sample. | —— | — | — |

| Number Weight (lbs.) Sex | Amount (cc.) of Triton, Day Given | Day of Aortic Freezing | Gross and Microscopic Observations | Serum Grossly Lipemic | Sacrificed Days $\bar{p}$ Triton | Days $\bar{p}$ Freezing |
|---|---|---|---|---|---|---|
| 53, 8.5 ♀ | 9.5 1 | 2 | Aorta - proliferated intima; slight inner medial lipid; medial necrosis.<br>liver - slight stainable lipid.<br>kidneys - no tubular lipid; cortical scars. | yes | 13 | 12 |
| 54, 8.5 ♀ | 9.5 1 | 2 | Aorta - without proliferated intima or medial necrosis.<br>heart - normal.<br>Extensive I.V.C. phlebitis; huge abdominal mass. | yes | — | — |
| 55, 7.9 ♀ | 9.0 1 | 2-sham | Venal caval phlebitis; renal cortical scars - no tubular lipid; no hepatic lipid; no aortic proliferation or lipid-periaortic arteriolar medial lipid. | yes | 7 | 6 |
| 56, 8.4 ♀ | 9.5 1 | 2-sham | Aorta - thickened and folded internal elastic lamina; very few foam cells.<br>liver - no fat.<br>kidneys - arterial intimal swelling; patchy parenchymal round cell infiltrates. | yes | 7 | 6 |
| 57, 7.5 — | 10.0 1 | — | Fatty liver. (22 hour lipemic serum used for electrophoresis.) | yes | 1 | — |
| 58, 7.5 — | 7.5 1 | — | Fatty liver. | yes | 1 | — |
| 59, 6.1 ♀ | (7.5 1 saline control) | 3 | Aorta - severely calcified media; subintimal profiferation without lipid.<br>Normal heart, liver, kidney. | no | (13) | 11 |

| Number Weight (lbs.) Sex | Amount (cc.) of Triton, Day Given | Day of Aortic Freezing | Gross and Microscopic Observations | Serum Grossly Lipemic | Sacrificed Days p̄ Triton | Sacrificed Days p̄ Freezing |
|---|---|---|---|---|---|---|
| 59 cont'd | | | lungs - congestion; subintimal pulmonary arteriolar swelling without lipid. Resuscitated after cardiac arrest. (2 accessory spleens) | | | |
| 60, 9.25 ♀ | (10 cc. 1 saline control) | 3 | Aorta - proliferation with some lipid deposition and foam cells; medial calcification. | no | (13) | 11 |
| 61, 7.6 ♂ | Corneas injected with 0.1 cc. supernat from 22 hr. pooled serum from WA-57, 58. | — | Sacrificed 8 days after corneal injection. Almost no corneal reaction except few isolated foam cells. | — | — | — |
| 62, 7.6 ♀ | Corneas injected with 0.1 cc. subnate from 22 hr. pooled serum from WA-57, 58. | — | Sacrificed 8 days after corneal injection. Almost no corneal reaction except few isolated foam cells. | — | — | — |
| 63, ~8.0 ♂ | 10.0 | — | Fatty liver. | yes | 4 | — |
| 64, ~8.0 ♂ | 8.0 subcutaneous | — | Fatty liver. | yes | 4 | — |

| Number Weight (lbs.) Sex | Amount (cc.) of Triton, Day Given | Day of Aortic Freezing | Gross and Microscopic Observations | Serum Grossly Lipemic | Sacrificed Days p̄ Triton | Sacrificed Days p̄ Freezing |
|---|---|---|---|---|---|---|
| 65, ~8.0 ♂ | Corneas injected with 0.1 cc. supernate from 4 day pooled sera from WA-63, 64. | — | Sacrificed 7 days after corneal injection; fine droplets of lipid halfway through outer cornea.<br>Corneas grossly milky. | — | — | — |
| 66, ~8.0 ♂ | Corneas injected with 0.1 cc. subnate from 4 day pooled sera from WA-63,64. | — | Sacrificed 7 days after corneal injection; fine droplets of lipid halfway through outer cornea.<br>Corneas only slightly cloudy.<br>Mederately well circumscribed region of foam cellular reaction. | — | — | — |
| 67 | 10.0 1 | — | Lost in animal room. | yes | — | — |
| 68, 8.75 ♀ | 10.0 1 | — | Diminished peritoneal and perinephric fat.<br>lungs - left upper and lower lob pneumonial, pulmonary arteriolar focal cellular infiltrate. | yes | 26 | — |
| 69, 7.75 ♀ | 10.0 1 | — | Abdominal wall absess.<br>lungs - left upper and lower lobe pneumonial, pulmonary arteriolar focal cellular infiltrate. | yes | 26 | — |
| 70, 8.0 ♀ | 10.0 1 | — | Abundant peritoneal fat.<br>lungs - bilateral lower lobe pneumonia; pulmonary arterioles have foam cells and proliferated intima.<br>(L)kidney - white 2 mm. cortical sear-superior pole. | yes | 26 | — |

| Number Weight (lbs.) Sex | Amount (cc.) of Triton, Day Given | Day of Aortic Freezing | Gross and Microscopic Observations | Serum Grossly Lipemic | Sacrificed Days $\bar{p}$ Triton | Sacrificed Days $\bar{p}$ Freezing |
|---|---|---|---|---|---|---|
| 71, 9.1 ♀ | 10.0 1 | — | Abundant peritoneal fat. heart and kidneys normal. lungs - bilateral lower lobe pneumonia; pulmonary arterioles have foam cells and proliferated intima. | yes | 26 | — |
| 72, 6.0 ♀ | 10.0 1 | — | lungs - bilateral lower lobe pneumonia; pulmonary arterioles have foam cells and proliferated intima. Abundant peritoneal fat; renal cortical scarring. | yes | 26 | — |

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